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SILVER-SCURF OF THE IRISH POTATO CAUSED BY SPONDYLOCLADIUM ATROVIRENS

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INTRODUCTION

Silver-scurf of the Irish potato (*Solanum tuberosum*), caused by *Spondylocladium atrovirens*, has been known in Europe since 1871, when it was discovered by Harz (6) on new potatoes in Vienna; but there is no record of its appearance in this country until mentioned by Clinton (4) in 1908. Notwithstanding its comparatively recent discovery, its general distribution in the eastern United States was shown by Melhus (7), 1913, who also raised the question as to its importance as a new potato disease in America, while its appearance in the Northwest was first reported in 1914 by Bailey (2) and later, in 1915, by O'Gara (8).

Reports of studies made by former investigators contain contradictory assertions, especially on the effect of this organism upon the host. It is evident, therefore, that further study of the symptoms, manner of infection, and physiology of the organism is desirable in order to understand more fully the significance of this disease, which has already become widely distributed in this country.

STUDIES OF THE FUNGUS

MORPHOLOGY

Spondylocladium atrovirens, one of the black molds, is classified according to Saccardo (9, p. 483) in the Fungi Imperfecti under the Dematieae. The genus Spondylocladium is characterized by its dark multiseptate conidiophores, which bear the many-celled conidia pleurogenously in the form of whorls.

Conidiophore and conidia formation can be studied either in hanging-drop or agar cultures. When the organism was cultured on agar plates

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held at room temperature, conidiophores and conidia appeared in 10 to 12 days, which indicates that *S. atrovirens* is one of the slow-growing fungi.

The conidia are formed first either at the apex or the distal end of the intermediate cells. Under certain apparently abnormal conditions, however, they appear at the ends of what seem to be ordinary branches of the mycelium, but in that case the character of the normal conidiophore is absent. The lowest whorls of conidia are borne about halfway between the base and the apex of the conidiophores, and the conidia are attached at the broad end (Pl. XLVI, fig. 2).

Germination of the conidia takes place by means of germ tubes. These are produced from either pole, generally from the distal or pointed end, as well as from any cell of the conidium, as observed by previous investigators. Germination in water occurs within 24 to 40 hours; and in a few days the somewhat hyalin, knoblike protrusion, which is characteristic of the early stages of germ formation, develops a multiseptate, branched mycelium which is of a much lighter color than the conidiophores, conidia, or portions of the old mycelium. This is very hyalin and continues so up to the time of conidiophore formation, at which time dark-brown, thickened cells are formed in different parts, and from these specialized cells are produced the many-septate, dark-brown conidiophores, which attain a length of 5 mm. and are perceptibly wider than the surrounding mycelium (Pl. XLVI, fig. 1).

Because of the wide variation found in the size of the spores, Appel and Clinton (4, p. 359) suggested the possibility of there being two species of the fungus—that is, a large-spore and a small-spore species. Several series of 18 measurements were made by the writer on conidia taken from tubers imported from Germany and tubers from various parts of the United States. A wide variation in dimensions occurred in the conidia from all the various tubers used in the experiment. The conidia taken direct from the surface of the tuber from Germany varied from 22 to 42μ (mostly 30 to 40) in length, 6 to 12μ (mostly 6 to 8) in width at greatest diameter, and were 4 to 8 (mostly 5 to 6) septate; conidia taken from the progeny of tubers from Maine grown in Washington, D. C., varied from 30.4 to 56.2μ (mostly 30 to 40) in length, 7.6 to 9.5μ (mostly 7.6 to 8.5) in width, and were from 4 to 7 septate; while the conidia taken from tubers from Rhode Island, West Virginia, Washington, D. C., Oregon, Washington, and Wisconsin averaged 32.6 to 40μ in length, 7.5 to 8.5μ in width, and were 5- to 7-septate.

In order to study more fully the variation of spore dimensions, several series of measurements were made on conidia produced from a single spore strain. The difference in dimensions obtained in this case ranged from 18 to 64μ (mostly 30.4 to 40) in length, and 7 to 8.1μ in width, and 5 to 6 septa.

From this it is apparent that, even though considerable variation in spore dimensions occurred on infected tubers from different localities, nevertheless an even greater variation resulted in the case of spores from a single spore strain. This shows that normally a wide variation exists, and consequently it does not appear necessary to form small-spore and large-spore species.

REACTION OF THE FUNGUS TO LIGHT

In order to secure a better knowledge of the relation of *S. atrovirens* to its environment so that its life history might be better understood, experiments on some of the physiological characteristics of this organism were conducted.

The reaction to light is of special interest in connection with the effect of storage conditions upon the development of the fungus on potatoes.

In this study the writer used the plate-dilution method, the conidia being sufficiently diluted on Lima-bean agar plates to be observed individually. Immediately after the plates were poured, each was wrapped in carbon paper, the entire dish being covered except an aperture from 1 to 2 cm. in diameter at the side, and the plates were then arranged with the apertures facing the light from the window.

The plates were examined at the end of three days and it was found that the mycelial branches developed on the side of the hyphæ farthest away from the window and that the majority of these grew in the opposite direction from the source of the light. The position of germ-tube formation does not appear to be influenced by the light, germination sometimes taking place from the side closest to the source of light; but as soon as the germ tube receives the heliotropic stimulus—that is, when it is a few millimeters long—it invariably turns away from the light, and subsequent mycelial development is formed on the side of the conidium farthest from the source of light. Instead of appearing at the center of the colony, therefore, the conidia are found at the margin exposed to the light, and at the end of 5 to 10 days the entire colony appears as if a gentle breeze had blown the hyphæ in one general direction away from the light (Pl. XLVI, fig. 3). These results also confirm Eichinger's (5) observations.

The reaction of this fungus to light in culture media demonstrated that it is negatively heliotropic. In view of the fact that infection of the tubers in the field takes place in the dark, negative heliotropism here does not obtain. In order to determine whether this heliotropic property favored tuber infection, artificial inoculations were made on tubers in the light. In this case no perceptible difference occurred, since infection appeared on all parts of the tubers alike.

REACTION OF THE FUNGUS TO MOISTURE

Like most fungi, *S. atrovirens* requires considerable moisture for development; but, owing to the absence of accurate instruments for

measuring the degree of moisture, only approximate data regarding moisture reaction can be given. It was noted in field studies that a higher percentage of infection occurred in the lower and more moist sections of the field than in the higher areas, and that in laboratory infection experiments the fungus develops best when the surface of the tuber is kept moist but not supersaturated. By placing tubers sufficiently near water so that a heavy film of moisture was constantly present, it was found that sporulation was inhibited to a greater degree on the side of the tuber near the water than on the opposite side, which indicates that excess moisture may check the growth of the fungus.

Although the fungus prefers moisture for growth, it can withstand drying without the entire loss of its virility. This was shown by the fact that transfers from agar cultures 16 months old continued to grow, although only a small percentage of the conidia germinated. Notwithstanding the fact that these cultures had been kept at room temperature and were dried to such an extent that simply a dry, brittle mass of media and fungus remained, both viable conidia and mycelium were found.

REACTION OF THE FUNGUS TO TEMPERATURE

Conidia in corn meal and oat agar and in water and naturally infected and artificially inoculated potato tubers were used in studies to determine the effect of temperature on *S. atrovirens*. In the case of media spore-dilution plates were prepared, the spores being sufficiently far apart so that individual colonies were retained. The same dilution was used on each plate and all were inoculated at temperatures ranging from 2° to 31° C. The water cultures were used in making hanging-drop preparations on Van Tiegham cells and in small Petri dishes, the spore suspensions in this case also being made in such manner that some of the spores remained on the surface, although germination occurred to a slight extent also beneath the surface. The naturally infected and artificially inoculated tubers were placed in pint bottles containing some pebbles and a few cubic centimeters of water, with a piece of cheesecloth extending from the contents of the bottle to its mouth, thus forming a moist chamber. These bottles were incubated in the same way as the media cultures.

In the eight series of Petri-dish cultures microscopic germination was noted at 3°, 4°, and 5° C., but no macroscopic colonies developed; at temperatures ranging from 6° to 28° macroscopic colonies were obtained, 21° to 27° being the optimum for abundance of growth; while at 30° or 31° no macroscopic growth was apparent (Pl. XLVII). These temperature limits for growth were confirmed by the water cultures, which were used as checks on the media cultures subjected to the highest and the lowest temperatures. In the case of three series of these water cultures which were subjected to a temperature of from $-5°$ to $-10°$ C. for four days and then brought to room temperature, 80 per cent of the

conidia germinated within 48 hours, and pieces of the mycelium in the cultures also showed growth. Agar culture and cultures on sweet-clover stems subjected to the same temperature also remained viable, as indicated by subsequent transfers, hanging-drop cultures showing that both conidia and mycelium retained their vitality.

In the test with naturally infected and artificially inoculated potatoes sporulation occurred on the former at temperatures ranging from 6° to 27° and on the latter at a range of from 12° to 27° C. In cultures on agar media and sweet-clover stems subjected to 35° and 50° further growth was inhibited at the former temperature, but the fungus remained alive after two weeks' exposure, while it was killed when subjected to 50° for three days.

REACTION OF THE FUNGUS TO MEDIA

Since *S. atrovirens* is a relatively slow-growing organism, tests were made with media of different grades of acidity with a view of facilitating growth in culture. The media used for this purpose were synthetic, Lima-bean, string-bean, oat, potato, corn-meal, and beef agar, all of which varied in reaction from +15 to −15 Fuller's scale.

Two plates each of these media equally diluted with conidia from the same culture were poured, and all were incubated at room temperature. Examinations of the colony development, including nature and extent of growth and sporulation, were made at 4-, 6-, and 12-day intervals and showed that *S. atrovirens* developed slightly faster on potato and Lima-bean agar than on string-bean, corn-meal, or oat agar; that growth was much retarded on beef agar; that mycelial development was very decidedly inhibited on synthetic agar; that sporulation occurred slightly sooner on oat agar than on the other agars; and that the hyphæ on fruiting remained lighter in color on Lima-bean and beef agars than on other agars.

The optimum reaction appeared to depend largely on the kind of medium. On potato agar no perceptible difference in growth appeared between +10 and −10, but mycelial development was much retarded at +15. On corn-meal agar only +1, 0, −1, −3, −5, and −15 reactions were run, because of the fact that hydrolysis took place when there was a higher degree of acidity. In this series +1 reaction was the optimum for growth, and in this case the mycelium became dark earlier than was the case in the minus reactions, owing possibly to the hydrolytic action of the acid on the media. On Lima-bean agar with +5 to −3 reactions the apparent growth of the fungus was not much changed, but with 5 to 10 and −3 to −10 reactions mycelial growth was perceptibly retarded. On beef agar optimum reactions ranged from 0 to +1, very little difference appeared in the colonies at +3 to −3, growth was gradually retarded at 5 to 15, and no colonies were macroscopically visible at the end of 10 days on reactions ranging from −5 to −15.

Besides this test of different reactions of the medium, a series of nutrition tests was conducted, a full nutrient agar, including carbon, nitrogen, oxygen, hydrogen, potassium, phosphorus, magnesium, sulphur, and iron, being used. With one exception each set of the media contained one element less than the full nutrient culture; in other words, the experiment was arranged as follows: (1) Check containing water agar, (2) full nutrient, (3) full nutrient minus nitrogen, (4) full nutrient minus potassium, (5) full nutrient minus phosphorus, (6) full nutrient minus magnesium, (7) full nutrient minus sulphur, (8) full nutrient minus iron, (9) full nutrient minus carbon, (10) full nutrient minus all minerals. Two plates of each kind of agar were inoculated with conidia and two with mycelium from the same culture of *S. atrovirens*, and all were incubated in the laboratory at room temperature.

Examinations at the end of 15 and 20 days indicated that sporulation occurred only on the plates from which sugar was omitted—that is, Nos. 1 and 9—the colonies on these plates being of a light color and spreading character and from 1.5 to 2.5 cm. in diameter and that no sporulation occurred on the plates from which sugar had been omitted, the mycelium in these being dark and densely compacted and only 0.75 to 1.25 cm. in diameter.

This preliminary study of the reactions of media on *S. atrovirens* indicates that neutral or slightly acid reactions are more favorable for the growth of this fungus; that the kind of medium determines the effect of higher reactions on this organism as shown by the alkaline reactions of beef agar compared with the same reactions of potato or the other agars; that compounds in one kind of medium may be formed which are seemingly toxic, whereas in a different kind of medium the same adjustment produces no such inhibitory effects; and that the presence of 5 per cent of cane sugar in a nutrient agar inhibited sporulation, but induced dark, heavy, compact mycelial growth, while the absence of sugar caused sporulation and a more spreading mycelial development.

HISTOLOGY

Studies were made to determine the relation of *S. atrovirens* to the potato. Both normal and affected material from the eye end of Irish Cobbler, Green Mountain, and Minnesota Triumph tubers badly infected normally and artificially was taken from the center and from the margin, that from the latter with and without lenticels or eyes. This material was embedded, sectioned, and stained according to ordinary cytological methods. From these studies it was evident that the mycelium may enter the tuber through the lenticels or between the lenticels through the epidermis.

After the fungus gains entrance the hyphæ invariably form within the cells, where they appear as a single branch of the mycelium; or they

may shorten and thicken to form a short and many-celled mass of hyphæ, from which the conidiophores subsequently arise. In severe cases of infection the cells appear to be disintegrated by the invasion to such an extent that only two or three instead of six or more cork layers remain above the living parenchyma. In experiments with potato roots grown under sterile conditions and inoculated with conidia and mycelium of the fungus, the mycelium grew on the surface, but did not penetrate the parenchyma, which indicates that the roots are less subject to infection than the tubers.

So far as the author has been able to determine, the fungus hyphæ confine their activity to the corky layers. In no case has it been found in the living parenchyma. This superficial infection causes a loosening of the corky and epidermal cell layers, so that these subsequently slough off. In this manner transpiration may proceed with greater facility and thus affect the parenchyma layers.

That *S. atrovirens* prefers this relatively heavy corky layer is further apparent from the fact that it grows very sparingly on the cut surface of the tubers where the loosened surface cells are invaded. Furthermore, its very limited presence on roots, stems, and stolons also indicates that it prefers the heavier, corky layers of the potato tuber.

EFFECTS OF THE FUNGUS ON THE HOST

The progress of the disease after tuber infection may be divided into two stages, the early and the late. In the former the infected areas are light-brown and have a glazed appearance, the latter characteristic becoming especially pronounced when the infected surface is moistened. Sometimes the margins of these areas are slightly fimbricated. The discoloration, which is found on newly infected tubers at harvest time, is often so inconspicuous as to pass unnoticed, even on close examination, unless the tubers are washed. When infected tubers are placed in moist chambers, the brownish areas become olive-colored, owing to the formation of conidiophores and conidia. The late stage is characterized by the shrinking and shriveling of the diseased areas and sloughing off of the epidermis and may be subdivided into two stages: The spot or patch infection (Pl. XLV, fig. 2) and general infection (Pl. XLV, fig. 1). In the former slightly sunken isolated areas on the surface show the shriveling, and late in the storage season these areas become shriveled and sunken.

In the case of general infection the entire surface is covered with infected areas and the epidermal and corky layers may shrink to such an extent that distinct folds or ridges appear. In the red-skinned varieties the color is completely destroyed. This again largely only mars the appearance and not their food value, but still they must be sold at a sacrifice. Potatoes stored under moisture and temperature

conditions favorable to sporulation often become so badly infected that they become a dull-black, the tubers having the appearance of having been dusted with soot. Several such bins were observed in Maine in May and June, 1914.

In case of slight infection in the field the infected areas are often found in isolated spots close to the stem end of the tuber. This was the case in practically every infected tuber harvested from the silver-scurf experimental plot at Caribou, Me., in the fall of 1914 and coincides with the observations of Appel and Laubert (1). While no reasons for this phenomenon are given by these investigators, from experiments and observations so far made it appears that infection is brought about through contact of the stem end of the young tuber with the infected mother tuber (Pl. XLVIII). This is indicated by the fact that in many cases where there was but slight contact only small areas about the point of the stolon attachment showed infection, while in the case of extensive contact infection was more widespread. It is further indicated by the fact that only one or two tubers closest to the mother tuber showed infection in counts made when the crop was about three-fourths grown, while in counts made later, after the conidia had become generally distributed, a large percentage of the tubers were infected.

Although infection appears to take place through the stem end, both stem ends and eye ends are subject to infection, general infection of both resulting from artificial inoculations.

In view of the fact that investigators like Bohutinsky (3) have attributed to *S. atrovirens* foliage symptoms such as leafroll, mosaic, etc., inoculations upon stems, stolons, and roots of the potato plant were made, both under field and greenhouse conditions. Two distinct procedures were followed: In one set of experiments viable spores were sprayed upon the stems, stolons, and roots; in the other virile mycelium was inserted into the inoculated portions. Checks were also run. Experiments in this order were run during 1914 and 1915, and in every case the inoculated plants behaved like the checks—viz, no perceptible infection occurred—showing again the inability of this organism to invade the vine tissues of the host.

METHODS OF DISSEMINATION

The fungus lives over by means of the mycelium, conidia, and sclerotia within the infected areas, so that under favorable conditions of moisture and temperature sporulation occurs and infection may spread even in storage. Not only do the infected tubers carry the disease to new sections, but they may carry it over from one season to another in the soil and in this way infect the new crop. This was the case in the author's field studies in Maine, viable conidia being found on the surface of

mother tubers taken on August 2, 1914, the date of the last examination, from an oat field at Houlton, in which they undoubtedly over-wintered in the soil. Many of these volunteer plants occurred in fields in which rotation had not been practiced, the deep snows which covered the ground the previous winter having protected the tubers.

Whether the fungus may live over in the soil from which the tuber host has been removed is not yet known, but that it may do so is not improbable, in view of what occurs in the case of fungi having a similar life history. Investigations to determine this point are now in progress.

Several series of experiments were undertaken to ascertain how readily *S. atrovirens* spreads from infected to healthy tubers and whether infection in this way might occur during the entire storage season. Inverted bell jars were used in these experiments to secure moist chambers which would hold a sufficient number of tubers for a satisfactory test and at the same time retain uniform moisture conditions. A wire rack of ¼-inch mesh was placed in each jar to support the potatoes and to prevent contact with the water in the jars, the inside of each jar was lined with blotting paper to conserve the moisture and prevent the entrance of excessive light, and the mouth was covered with window glass. Four varieties of potatoes were used: Rural New Yorker, Green Mountain, Irish Cobbler, and Bliss Triumph. A spore suspension of conidia which had been grown in pure culture on sweet-clover stems for four weeks was sprayed on the tubers with an atomizer, and for several days thereafter water was sprayed into the jars with the atomizer to keep the air saturated. A similar lot of healthy tubers was arranged as a check.

The first series was begun at Houlton, Me., on March 26, 1914; and within three weeks the entire surface of the inoculated tubers was covered with dark-brown conidiophores and conidia, while the checks were free from infection. Additional tests were made at Caribou, Me., on July 20, 1914; Washington, D. C., in December, 1914; Madison, Wis., on March 25, 1915; and Presque Isle, Me., on August 2, 1915. In each case infection occurred within three weeks after inoculation.

Similar infection experiments were conducted upon young tubers just harvested, as well as upon tubers still attached to the vines. In case of the tubers attached to the vines the soil was removed and a spore suspension was applied with an atomizer, whereupon the tubers were again covered with earth. Checks also were made. In each of these tests infection appeared upon tubers varying in diameter from 1 and 2 cm. to full-grown tubers (Pl. XLVII). Checks showed no infection.

From these results it is apparent that infection from *S. atrovirens* may take place at any stage in the development of the tubers and at any time throughout the storage season.

METHODS OF CONTROL

Melhus (7) found in laboratory experiments that neither double strength of mercuric chlorid (1:500) nor formalin applied for longer than the ordinary periods would completely inhibit the development of *S. atrovirens* on the potato and that both injured the tubers to such an extent that germination was decidedly inhibited. He also found that in many cases sporulation was inhibited on the surface of infected tubers treated with solutions of mercuric chlorid heated by a method devised by him for heating the solution for brief periods at temperatures near the thermal death point of protoplasm.

In view of these results, field tests were conducted during 1914 and 1915, both in Maine and at Norfolk, Va. Infected tubers were treated in double strength and heated solutions of mercuric chlorid. In Maine the treated tubers were planted on virgin soil.

As noted in Table I, the temperature fluctuated slightly, owing to the lower temperature of the tubers than that of the solution in which they were immersed. This table indicates that there was a decrease in the percentage of infected progeny in the treated rows as compared with the check. However, in no case was there a complete control of the infection. Similar tests in 1915 also indicated that even though silver-scurf may be inhibited to some extent; nevertheless, no treatment served as a complete control.

TABLE I.—*Effect of warm solution of mercuric chlorid on silver-scurf of the Irish potato*

Row No.	Strength of solution.	Temperature of solution.	Time of immersion.	Number of hills.	Number of hills infected.	Per centage of hills infected.	Average per centage infected hills in rows 14, 15, and 16.	Weight of healthy tubers.	Weight of infected tubers.	Percentage of infected tubers.	Average per centage of infected tubers in rows 14, 15, and 16.
	Per ct.	°C.	*Min.*					*Pounds.*	*Pounds.*		
13	.....	Control.	.....	78	40	51. 28	.....	85	11. 25	11. 67	.....
14	0. 2	47–52	5	63	29	46. 03	.....	84	10	10. 13	.....
15	. 2	47–48	5	93	6	6. 45	21. 04	102	1	. 98	4. 91
16	. 2	45–49	10	75	8	10. 66	.....	75	2. 5	3. 21	.....

In October, 1914, four pecks of tubers infected with S. *atrovirens* were subjected to a 1 to 1,000 solution of mercuric chlorid ranging from 45° to 53° C. for four minutes with a view of ascertaining the effect of treating infected tubers with that solution before storing. After treatment the tubers were placed in new muslin peck sacks and part of the lot stored at Caribou and part at Washington. At the same time separate lots of untreated infected and clean tubers were stored. On exami-

nation of these lots in June, 1915, the fungus was found fruiting on both treated and untreated infected tubers, but no infection was found on the untreated clean tubers.

As the treatments described do not absolutely control silver-scurf and as clean tubers only escaped infection, it is evident that disease-free seed should be selected in the fall and should be kept from contact with infected tubers in storage. Moreover, in view of the inhibitory effect of very low temperatures on the development of the fungus, the tubers should be stored at the lowest temperature permissible.

SUMMARY

A study of silver-scurf of the Irish potato, caused by *Spondylocladium atrovirens* Harz, shows that, notwithstanding the wide range in spore dimensions, which led certain investigators to believe there might be a large-spore and a small-spore species in this country, there is but one species, as proved by the fact that conidia ranging from 18 to 64μ were produced by a single spore culture.

S. atrovirens is negatively heliotropic. This, however, does not materially influence tuber infection in nature.

Severe drying of the conidia and mycelium in agar culture at room temperature does not kill the fungus.

S. atrovirens withstands a wide range of temperature. Its growth is inhibited at 2° to 3° C., but it is not killed at − 10°. Its optimum temperature is 21° to 27°, maximum 30° C.

Optimum reaction to media varies with the kind used, neutral to slightly acid reactions being most favorable to the development of the fungus. Five per cent of cane sugar in nutrient agar inhibited sporulation.

The fungus enters the tuber through the lenticels or the epidermal layers between the lenticels. The mycelium invades and disorganizes the epidermal and corky layers, leaving in bad cases only one or two instead of six or more layers, thus apparently accelerating transpiration.

The disease may be carried from place to place by infected tubers, in which it lives over from one season to another, or to the succeeding crop by the infected tubers which remain in the field over the winter.

Under favorable moisture and temperature conditions potatoes may become infected throughout the entire storage season. Both old and young tubers are subject to infection.

Inoculations on living stems, stolons, and roots in the field and laboratory experiments produced no infection.

Warm solutions of mercuric chlorid have a more toxic effect on *S. atrovirens* than cold solutions.

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PLATE XLV

Fig. 1.—Potato tubers showing shriveling and a silvery appearance caused by *Spondylocladium atrovirens*.

Fig. 2.—Tuber naturally infected by *S. atrovirens*, showing the segregated area type of infection, a condition developing in some cases later in the storage season.

Fig. 3.—Immature potato tuber artificially inoculated with conidia of *S. atrovirens*, July, 1913, at Houlton, Me. Infected area covered with dark-brown tufts of conidiophores and conidia. Infection was effected in a moist chamber at room temperature.


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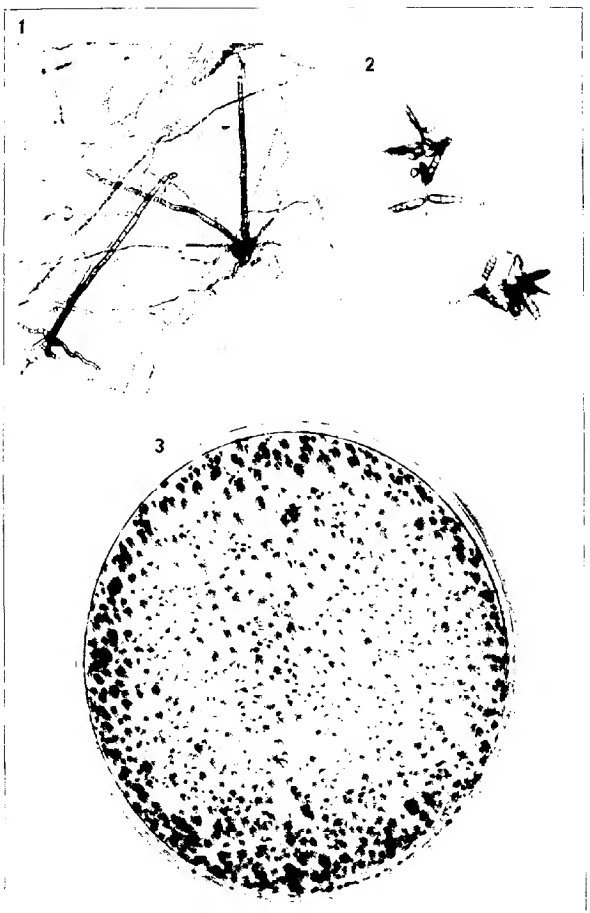
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PLATE XLVI

Fig. 1.—Photomicrograph of *Spondylocladium atrovirens* on corn-meal agar, showing method of development of conidiophores and conidia in the early stages.

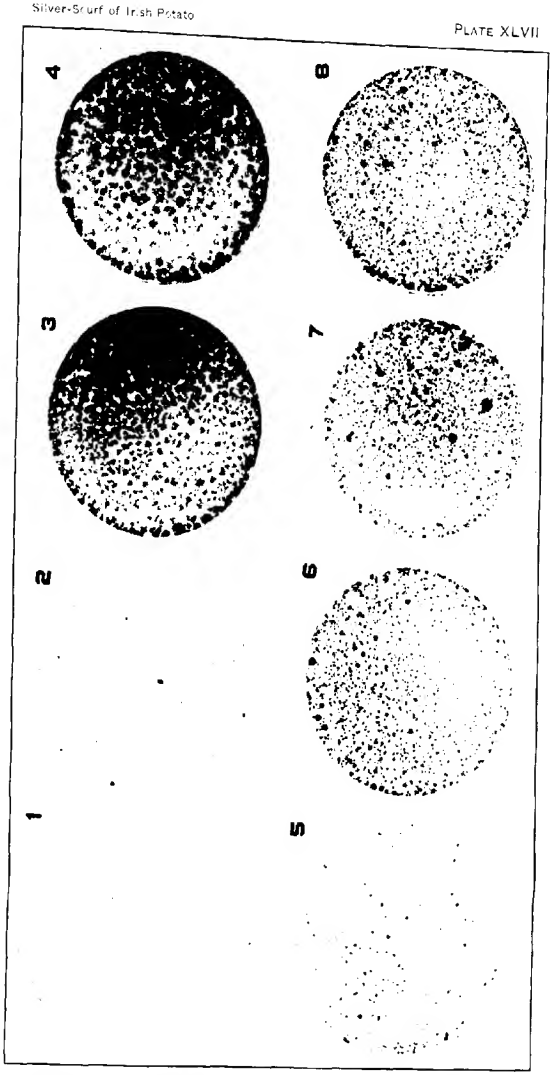
Fig. 2.—Photomicrograph of *S. atrovirens* in hanging-drop culture, showing development of conidiophore and conidia in mature stages.

Fig. 3.—Negative heliotropism of *S. atrovirens* on corn-meal agar exposed on one side to daylight from April 8 to April 24, 1915, in laboratory at room temperature.

PLATE XLVII

Effect of temperature upon mycelial development of *Spondylocladium atrovirens* in pure culture on corn-meal agar at end of four weeks.

Petri Dish No.	Temperature (°C.)	Petri Dish No.	Temperature (°C.)
1	3	5	10
2	5	6	15
3	24	7	16
4	27	8	13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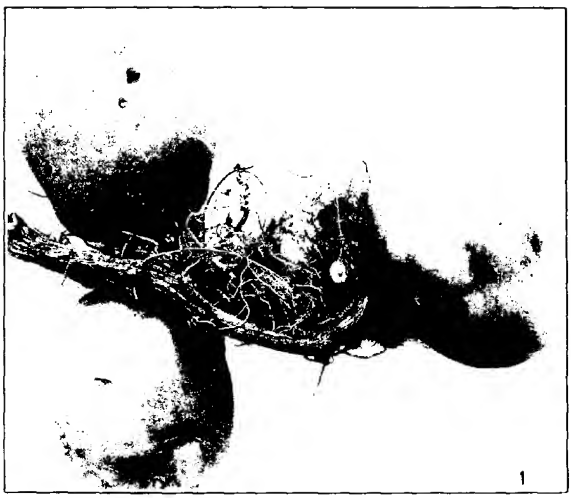
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2

PLATE XLVIII

Contact infection. A part of the new tubers becoming infected with *Spondylocladium atrovirens* by means of contact with the infected mother tuber. In this case it is a distinctly stem-end infection. Harvested on September 19, 1915, at Presque Isle, Me.

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WOOLLY PEAR APHIS[1]

By A. C. BAKER, *Entomological Assistant*, and W. M. DAVIDSON, *Scientific Assistant*, *Deciduous Fruit Insect Investigations, Bureau of Entomology*

INTRODUCTION

For some years a species of Eriosoma has been known to attack pear roots in California. It has, however, been considered to be the woolly apple aphis, *Eriosoma lanigerum* Hausmann, since both in habit and in structure the two species somewhat resemble each other. To the species on the pear, which, after careful study, proves to be undescribed, the name *"Eriosoma pyricola"* is herein given, and a brief account of the species is attempted.

HISTORY OF THE INSECT

Mr. Frank T. Swett is authority for the statement that the woolly pear aphis has been in California for more than 20 years. Ten years ago he says the species ruined about 2,000 French seedlings in one block, while occasional apple seedlings, planted along with them, made normal growth. Attention has frequently been called to the immunity of apple seedlings planted close to infested pear seedlings in nurseries and orchards.

During September and October, 1897, Mr. Theodore Pergande received specimens of a species of Eriosoma on pear roots from Prof. F. M. Webster, of Wooster, Ohio. Through the kindness of Mr. Pergande we have been able to examine these specimens, and they prove to be identical with our California material. It is quite possible, therefore, that the species may be present in other parts of the country, notably in Oregon. It is noteworthy that the Ohio specimens were taken from roots of pear stock received from France the preceding spring.

The species occurs over practically all the pear sections of northern and central California, and in some regions is a very destructive pest. To entomologists the extent of its presence has been known only for the last three or four years, but reports from orchardists and field observers indicate that it has been parasitic upon pear roots for a much longer period.

HABITS OF THE INSECT

The insect works entirely underground. The species that has been found feeding on the aerial portions of Nelis, Easter Beurré, and other pears is the woolly apple aphis, *E. lanigerum*. The woolly pear aphis

[1] What is probably the same species has been treated as a pear pest in California under the name *Eriosoma lanigera* by Geo. P. Weldon. (The woolly aphis as a pear pest. *In* Mo. Bul. State Com. Hort. [Cal.], v. 4, no. 9, p. 441-444, fig. 94-95. 1915.)

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appears to attack the roots of all types of pears, and it is especially injurious to the French wild stock so largely used in California as a stock for the Bartlett. Quince roots are fed upon, but much less freely, and the quince may be credited with a considerable degree of immunity. The Kieffer stock is attacked, but it is possible that Japanese stock may show immunity to a satisfactory degree. Observations to date indicate that both these stocks are more resistant than that from France. It should be said that the individual plants of the wild stock from France vary greatly, and there appears to be among the plants some variation in intrinsic vigor or in power to resist the woolly aphis. However, the majority of the imported seedlings show no satisfactory evidence of a power of resistance, and a different stock is very desirable.

The insect works especially upon the smaller fibrous rootlets and may be encountered on any such rootlets within the topmost 3 feet of soil and perhaps deeper. Infestations are usually heavier on the rootlets near the trunk, but frequently the aphides are as abundant 10 or 12 feet from the stem. In a badly infested orchard the soil on being overturned may in places be found to be white with the wool and skins of the insects. The aphides attack less frequently larger roots up to ¼ inch in diameter and sometimes settle on still larger roots or on the main stem where abrasions have set up a callus growth. They often colonize the underground portions of sucker growth, feeding on the succulent stalks. After the insects have forsaken a rootlet, fungi sometimes appear and complete its destruction.

This method of feeding upon the fibrous rootlets is somewhat analogous to the habits of the grape phylloxera (*Phylloxera vitifoliae* Fitch) on the resistant types of grapevines in that chiefly the smaller rootlets are attacked. It is directly opposed to the habits of the woolly apple aphis and of the grape phylloxera on nonresistant types of vines, for both these insects feed upon the larger roots and cause the formation of tuberlike lesions. The woolly pear aphis rarely forms any perceptible lesions, but it destroys great numbers of young rootlets, especially in late summer and autumn. In old trees this sometimes results in a dwarfing of growth and in a generally unthrifty appearance and condition. The majority of old infested trees do not show evident injury ascribable to the aphis, although it is presumable that they are suffering to some extent. They remain thrifty on account of their intrinsic vigor. In many instances where old trees were showing injury, extra cultivation of the soil and better irrigation practice resulted in the establishment of thrifty conditions, even though this method did not appear to reduce the numbers of the aphis. The effect on the crop is hard to estimate and can not be satisfactorily specified, but in general it is such as may result from the diversion of the flow of sap in the tree.

With trees under 4 years of age, conditions of injury are different. Heavy infestation of a tree of weak vigor or resistance may result in the death of the tree. Badly stunted growth and the early falling of foliage are characteristic of aphis injury on young trees. Injury and death are due to heavy summer and autumn infestations on the fibrous rootlets and to the inability of the tree to replace the destroyed roots quickly enough to afford plant food for the vegetative portion. Frequently the trees are saved and relief comes from the production in the fall months of a high percentage of migrants which leave behind them for the winter only a small infestation of wingless individuals; and since the aphides increase but slowly in spring, the tree is enabled to send forth new rootlets without danger of having them rapidly destroyed. Sometimes young trees in no wise stunted have been observed to cast their leaves prematurely, and upon examination have been found to be heavily infested with the aphis. It would appear from the absence of stunted growth that these trees did not have, or were not adversely influenced by, an infestation until their summer growth was about completed, and that the simultaneous destruction of feeding rootlets cut off the flow of sap suddenly. The fact that trees were stunted was an indication that the injurious effects of feeding by the aphides were felt earlier in the season.

In addition to trees noticeably stunted and others prematurely defoliated are found still others which show no external evidence of infestation and yet upon examination prove to be heavily infested. This phenomenon is frequently noticeable among young trees or in nursery rows, and hints at a power of resistance.

In orchards and districts where conditions favor large productions of winged forms, or migrants, spring and early summer infestations are small, denoting that few insects passed the winter on the roots. After the month of June, however, such infestations multiply rapidly and become very large by September, the month in which the fall migrants are produced in greatest abundance. After September there remain small wingless colonies which increase but little until the summer following. The winged forms are produced in abundance on heavy dry clay soils which crack in summer and autumn. Irrigated orchards produce them in smaller numbers than those that receive no moisture from May to October. On loam, silt, and light-clay soils the winged forms are much less abundantly produced. On such soils the infestation remains largely or wholly wingless the year around, and the conditions are generally unfavorable to such heavy infestations as occur on the heavy clays. The aphides appear to lack freedom of movement, and frequently their colonies are unable to increase perceptibly through summer. Occasionally the wingless infestations are severe the year round; where this is so, in the early part of the year there is caused a considerable stunting of growth and more or less

weakening, unless the trees can put out plenty of new rootlets to replace those injured and destroyed. This condition has been noted especially on light-clay soils where poor cultivation was employed.

SPREAD OF THE INSECT

In nurseries under favorable conditions the spread of the insect may be rapid. A half-acre pear nursery examined on June 9, 1915, failed to show infestation, though the aphis was probably present. When visited four months later, on October 16, it was found that more than half the trees examined were infested, some quite heavily. In large orchards where the soil is permeated throughout with rootlets the aphis doubtless is very easily diffused through the soil. In young orchards conditions indicate that not much spread takes place from tree to tree. Infested young orchards generally point to the nursery as the source of infestation, but the possibility of infestation through the winged forms, or migrants, must be considered. A knowledge of the full life cycle of the insect alone can clear up this point.

BIOLOGY AND DESCRIPTION OF THE INSECT

The wingless individuals live chiefly on the small rootlets and less frequently on roots and the underground portions of the sucker growth. They are always somewhat elongate and are for the most part pale yellowish red, but they may vary from a pale pink or yellow to deep red. They are rather sparsely clothed with long, curling, woolly, or cottony filaments, of which there are four or six on each segment. Toward the end of each instar these filaments are longer than the body—often three times as long. There is a sparse whitish powder on the body, more abundant at the caudal end. The cornicles appear as dusky-rimmed pores. The young are pale yellowish red and elongate.

The pupæ develop on the same portions of the tree as the wingless forms. They are very elongate in form and are clothed as are the wingless. The wing pads are inconspicuous and are white or light gray. As a rule pupæ on a rootlet develop almost simultaneously. The winged forms issue together, after which the narrow, elongate, cast pupal skins are conspicuous in little heaps, and are easily distinguishable from those of the wingless forms.

In the Walnut Creek district pupæ and winged migrants were collected in appreciable numbers from August 25 to November 17, and as late as December 22 a nymph was found. These forms were most abundant in September, and this observation apparently holds true for other localities in California. Wingless colonies collected at San Jose, Cal., on June 10 and thereafter, kept in Petri dishes with moist sand in a cellar, produced pupæ on July 20 and migrants from July 24 to August 7. This appeared to be abnormally early in the year for the production of winged forms, and

it may be that the environment and conditions hastened it. Under favorable conditions of soil the migrants were produced in great abundance on both young and old pear trees. In many cases, especially on young trees, it appeared that fully 90 per cent of the aphides observed at one time were pupæ, and in other instances observations in October and later after the winged forms had departed indicated that almost the entire infestation had developed into migrants. On old trees there remained on the average a larger residue of wingless forms. On unfavorable types of soil the winged forms are produced in far less abundance. It appears to be a rule that the heavier and drier the soil the larger the percentage of pupæ developing. It sometimes happens that the migrants are unable to rise to the surface of the ground and become imprisoned in pockets in the soil. In one instance two living sexual females were found in such a pocket beside dead migrants.

The winged forms have been noticed on pear foliage and on the trunk, but with one exception [1] no deposition of sexes has been observed on the pear. On cork and American elms (*Ulmus* spp.) migrants were observed to deposit the sexes in cracks in the bark and on the lower surface of leaves. In one instance the migration from a nursery of pear trees to a group of young elms 200 yards distant could be traced. The migrants fly readily and strongly and are stimulated by the sun's rays, being more active on warm than on cool days. On the elms they were more abundant on trees with rough bark than on the smooth-barked plants.

The migrants vary considerably in size. They are rather elongate, shining black or dark green, with a tuft of white wool on the caudal segment; otherwise, there is no flocculence. The lower surface is dark green, sparsely powdered at the sutures. The antennæ, eyes, and a portion of the legs are black. The base of the femora and the middle portion of the tibiæ are yellowish brown or amber. The wings have narrow black veins and a greenish blue stigma. The wing insertions are sometimes brown, but are more often yellowish. In recently molted individuals there is sometimes a smoky-brown patch at the base of the fore wings.

To obtain the sexes, migrants were confined in stender dishes and in small rubber cells mounted on microscope slides with cover glasses as lids. Some were kept in a lighted room in which the temperature varied very considerably, at times rising to 75° and at other times falling to 55° F. Others were kept in a dark cellar where the temperature varied but little and averaged about 61° F. Under cellar conditions the migrants deposited more sexual forms than under the conditions obtaining in the room. Some of the dishes were kept dry and others moistened to different degrees. In the moistened dishes the sex pro-

[1] In August, 1911, at San Jose, Cal., a migrant was noticed depositing sexes on the upper surface of a pear leaf.

duction was better than in the dry ones, although too much moisture prevented the sexual forms from freeing themselves from the pellicles. Whether the migrants had flown or not did not seem to bear any influence on the deposition of the sexual forms. In most of the dishes more than half of the sexed forms were not extruded, but died unborn. In the rubber cells five-eighths of an inch in diameter and three-sixteenths of an inch in height the migrants did best singly, while the larger stender dishes provided space for a number. In all the dishes pieces of pear or elm bark were provided, but the migrants rarely deposited the sexes on these, nearly always extruding them on the filter paper also provided. It frequently happened that the sexes after having been extruded became entangled with the wings or legs of the parents or with each other. The sexes were deposited in rapid succession. The migrants rarely lived beyond three days after they were placed in the dishes, whether they deposited sexual forms or not. None lived longer than six days. They died immediately after the sexes had been extruded and very few deposited their full complement.

All the sexes deposited were not noted; but about four-fifths of them totaled 109 individuals, of which a little over half (58) were females. Only a few matured, and the majority died unmolted. Undoubtedly the cause of this was the abnormal condition of the environment. However, it appears to be proved that the sexes are produced in about equal numbers, and observations in the field corroborate this. Four fall migrants dissected on October 27 and 28 had contained, respectively, 5, 7, 8, and 9 young. In the dishes not more than seven sexes were ever dropped by an individual. The number of males and females deposited by individual migrants was found to range from seven females and no males to five males and one female. Probably a larger series would have furnished a migrant producing only males. As a rule the production of sexes was about evenly divided between male and female.

The sexes have no woolly covering such as that occurring on the sexes of *Eriosoma lanigerum*, but are bare and shining. The female, however, at the time of depositing the winter egg, has a patch of short white wool on either side of her body and with this she contrives to clothe partly the winter or impregnated egg. The sexes are active, the male especially so, both immediately after extrusion and following the casting of their fourth and final skin. Between casting their first and fourth skins they remain inactive unless disturbed. Normally they seek crevices in the bark, but in the dishes they frequently molted on filter paper or on the sides and floor.

The sexes mature in from 7 to 11 days and molt four times—that is, about every other day. Being beakless, they take no food.

The males are smaller than the females, the latter being enlarged by reason of the egg within the body. The male at first is light green, with

hyalin antennæ and legs and black eyes of three facets. The insect becomes darker with age and the mature individual is dark olive-green, sometimes tinted with lilac or purple, the central part of the abdomen being darkest. The male is always narrow in shape. The female varies in color from a light orange to a dark red. The eyes and appendages are as in the male. The majority are orange or a light crimson-lake. They are much stouter than the males and are longer and stand much higher. A mature female measured alive was 0.67 mm. long by 0.33 mm. in maximum width. A mature male was 0.43 mm. long by 0.21 mm. in maximum width.

Copulation occurs as soon as the sexes are mature. It appears that unless the female is fertilized directly after she has cast her last skin she will fail to deposit the winter egg. The male may live at least a week after he is mature, but apparently he can exercise the sexual function only immediately after he has cast the last skin. The females deposit the impregnated egg immediately after copulation, and after its deposition they may live for a day or two at the most. The winter or impregnated egg is laid normally in crevices or scars of the bark of the elm. In the dishes it was laid sometimes on the outside of the bark, and both elm and pear bark were used. It was never laid elsewhere than in the bark. The egg measures about 0.444 mm. by 0.225 mm., is short oval, reddish yellow, and shining. The end first extruded is reddish and bare, while the other extremity is yellowish and usually covered with short white wool provided by the female. Winter eggs were deposited in dishes between October 15 and November 12. Undoubtedly they occur in nature as early as September 5, and may be laid as late as the middle of November. Toward the end of October some were collected under the bark of elms under observation. Table I is a comparison of the biology of *Eriosoma pyricola* with that of *E. lanigerum*.

TABLE I.—*Comparison of biology of Eriosoma pyricola with that of Eriosoma lanigerum in California*[1]

Eriosoma lanigerum on apple and varieties of pear.	*Eriosoma pyricola* on pear.
Aerial and radical.	Radical only.
Attacks trunks, branches, and twigs; causes knotty swellings on roots.	Attacks chiefly fibrous rootlets; rarely causes lesions; occasionally settles on larger roots.
Fall migrants rarely abundant; apparently not influenced by conditions.	Fall migrants very abundant under favorable conditions.

[1] The full cycle of these species has not been worked out in California, but there appear to be no records of spring generations of *E. lanigerum* observed on elm.

The fall migrants of *E. pyricola* may be distinguished from those of *E. lanigerum* and *E. americanum* as shown in Table II.

TABLE II.—*Comparison of the fall migrants of Eriosoma pyricola, E. lanigerum, and E. americanum*

E. pyricola.	*E. lanigerum.*	*E. americanum.*
Stigma short, greenish blue.	Stigma somewhat elongate, yellowish or gray.	Stigma elongate, gray.
Veins narrow without brown margins.	Veins narrow and without brown margins.	Veins broad, with brownish margins.
Body naked except for caudal segment.	Body with some woolly clothing.	Body with slight woolly covering.
Distal sensoria of antennal segments V and VI with fringe.	Distal sensoria of antennal segments V and VI without fringe.	Distal sensoria of antennal segments V and VI without fringe.

The new species is easily distinguished from *E. ulmi* Linnæus from the fact that segment V bears prominent transverse sensoria. The wingless forms can be distinguished from those of *E. lanigerum* by the structure of the compound wax pores, and the winged forms by the antennæ. The winged forms of *E. pyricola* are remarkably like those of *E. lanuginosa* Hartig. The proportions are almost exactly the same. The only difference seems to be the fringing of the sensorium on segment V. The wingless forms and the pupæ have the prominent wax pores figured. No such pores occur in our specimens of *E. lanuginosa*, but very similar ones do occur in *E. ulmi*. At first it was thought that two species were present in the collected material, but careful rearing experiments by the junior writer have shown the connection between all the forms. It does not seem probable that such prominent wax-secreting structures would be present in one form of the species and not in all forms.

Eriosoma pyricola, n. sp.

WINGLESS VIVIPAROUS FEMALE.—General form elongate. Antennal segments in length as follows: I, 0.048 mm.; II, 0.048 mm.; III, 0.1 mm.; IV, 0.04 mm.; V, 0.048 mm.; VI, 0.064 mm. (unguis, 0.032 mm.); segments armed with hairs (fig. 1, *E*), which are considerably longer than those met with in *lanigerum* (fig. 1, *D*), and with a large distal fringed sensorium on segments V and VI, as well as some smaller ones on VI. Compound wax pores very prominent and circular (fig. 1, *I*), those on the abdomen containing about 20 cells. Abdomen sparsely covered with hairs about 0.16 mm. long; cornicles circular, their rims more heavily chitinized on their inner margins than elsewhere. Wax reservoir apparently present as in *E. lanigerum* (visible as a clear yellow area in mounted specimens). Hind tibiæ about 0.44 mm. long; hind tarsus, 0.112 mm.; rostrum extending beyond the second pair of coxæ. Length, 1.92 mm.; width, 0.96 mm. The hairs on the antennæ of the young are especially prominent (fig. 1, *J*).

Young forms yellowish pink, older ones pink to red. Antennæ, legs, and labium dusky; eyes dark red, very minute.

INTERMEDIATES.—In the collection, Q. 6399, are a number of specimens which would be taken at first glance for wingless viviparous females. A careful study, however, proves them to be intermediates. No trace of wing pads can be found, but the eyes clearly show the intermediate nature of the specimens. In the normal wingless

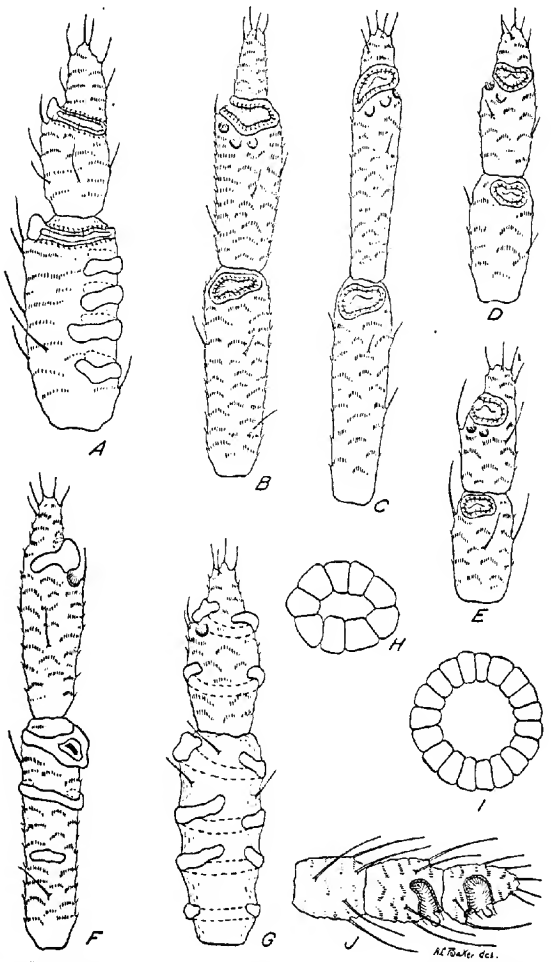

FIG. 1.—Comparative structure of antennæ and wax pores of *Eriosoma* spp.: *A*, distal segments of antenna of winged viviparous female of *E. pyricola*; *B*, distal segments of antenna of winged viviparous female of *E. ulmi*; *C*, distal segments of antenna of wingless viviparous female of *E. americanum*; *D*, distal segments of antenna of wingless viviparous female of *E. lanigerum*; *E*, distal segments of antenna of wingless viviparous female of *E. pyricola*; *F*, distal segments of antenna of winged viviparous female of *E. americanum*; *G*, distal segments of antenna of winged viviparous female of *E. lanigerum*; *H*, compound wax pore of *E. lanigerum*; *I*, compound wax pore of *E. pyricola*; *J*, distal segments of antenna of first instar wingless viviparous female of *E. pyricola*.

forms the eyes are composed of three facets and are very minute, whereas in these specimens the eyes are large and composed of numerous facets, thus approaching the compound eyes of the winged form. All other characters met with are those of the wingless viviparous female.

PUPA.—Antennal segments in length as follows: I, 0.048 mm.; II, 0.064 mm.; III, 0.192 mm.; IV, 0.064 mm.; V, 0.08 mm.; VI, 0.08 mm.; segments armed with hairs and sensoria as in the wingless female. Wing pads about 0.64 mm. long. Compound wax pores similar to those of the wingless females. Hind tibia, 0.432 mm.; hind tarsus, 0.128 mm. Body with long hairs as in the wingless form. Length, 2.32 mm.; width, 0.96 mm.

Pinkish, with a brick-red diffusion; wing pads whitish yellow; wool sparse, erect.

WINGED VIVIPAROUS FEMALE (FALL MIGRANT).—Antennal segments in length as follows: I, 0.048 mm.; II, 0.064 mm.; III, 0.432 mm.; IV, 0.112 mm.; V, 0.112 mm.; VI, 0.08 mm. (unguis, 0.032 mm.); segments I and II armed with a few hairs; segment III armed with about 20 transverse sensoria, which extend a little over halfway around the segment as in *E. lanigerum*, the dorsal side of the segment armed with numerous prominent hairs; segment IV similar to segment III and armed with four or five transverse sensoria; segment V (fig. 1, *A*) armed with three or four transverse sensoria and a distal fringed sensorium, a few hairs, and many rows of setæ; segment VI similar to segment V, but without transverse sensoria. The fringed sensorium at the base of the unguis varies in shape. Forewing somewhat similar to that of *E. americanum;* stigma short and rounded at the distal extremity. Hind tibia, 0.88 mm.; hind tarsus, 0.128 mm. Form elongate; length, 1.76 mm.; width, 0.72 mm.; forewing, 2.4 by 0.88 mm. Without wool.

Dark brown or very dark green. Base of femora and tibiæ yellowish gray. Stigma bluish gray. Abdomen shining.

Described from wingless females, intermediates, pupæ, and winged viviparous females in balsam mounts.

Type: Cat. No. 20083, U. S. National Museum.

PATHOLOGICAL HISTOLOGY OF STRAWBERRIES AFFECTED BY SPECIES OF BOTRYTIS AND RHIZOPUS

By NEIL E. STEVENS,
Pathologist, Fruit Disease Investigations, Bureau of Plant Industry

INTRODUCTION

The fungi causing rots of strawberries (*Fragaria* spp.) in transit from the Southern States have been under investigation by Dr. C. L. Shear, Mr. R. B. Wilcox, and the writer for the past two years. From the first it has been apparent that two species were chiefly responsible for their decay during shipment and on the market. These were *Botrytis (cinerea?)* and *Rhizopus (nigricans?)*.[1] The effect of these two fungi on ripe strawberries is strikingly different. Berries injured by *Botrytis* sp. show a characteristic dryrot—that is, they retain their shape, shrivel somewhat, and no leaking of juice is evident; whereas berries rotted by *Rhizopus* sp. quickly flatten out, with the loss of a large amount of juice. Such berries are characterized as "leaks" by growers and dealers.

F. L. Stevens[2] has already recognized a species of Rhizopus as the probable cause of leak. He, however, considers (p. 950) that *Botrytis* sp. "is the primary cause of the molding, that the *Botrytis* initiates the decay, opening the way to such other saprophytes as may be present; of such saprophytes, *Rhizopus* is by far the most prominent and most abundant." In order to determine if possible the relations of these fungi in rotting strawberries and in particular what differences exist in their method of attack on the fruit, a study of strawberries affected by these fungi was undertaken.

EXPERIMENTAL METHODS

The strawberries examined were chiefly of the Klondike variety grown in Louisiana during the season of 1915. Berries of other varieties grown in South Carolina and at Arlington Experimental Farm, Va., in 1915, as well as the Missionary and Klondike varieties from Florida in 1916, were used for comparison. Naturally infected berries as well as sound berries inoculated with spores and mycelium from pure cultures were used in both cases.

The material was fixed in a solution of equal parts of absolute alcohol and glacial acetic acid. This fluid penetrates very rapidly, so that whole strawberries are satisfactorily fixed. In the case of large berries,

[1] In the present uncertainty regarding the taxonomy of these genera it seems unwise to attempt a definite determination of the species. Permanent mounts of the material described are preserved, however, and cultures of the species considered are retained for further study.

[2] Stevens, F. L. A destructive strawberry disease. *In* Science, n. s., v. 39, no. 1017, p. 949–950. 1914.

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however, the ends were cut off to hasten penetration. Strawberry cells are so large that rather thick sections, from 10 to 20μ, were found most desirable. The walls of the strawberry cells and of the fungus hyphæ are so similar that differential staining was rather difficult. The best differentiation was secured by a combination of methylene blue and clove-oil eosin, using a water solution of tannin as a mordant. This method was suggested to the writer by Mr. Charles S. Ridgeway, of the Bureau of Plant Industry. The hyphæ, however, are so large as to be easily distinguished when the sections are properly stained with the more permanent stains, as safranin, Delafield's hematoxylon, or even Bismarck brown.

RESULTS OF INFECTION OF STRAWBERRIES BY BOTRYTIS SP.

Botrytis sp. has long been a favorite subject for the investigation of the relations of host and parasite. The somewhat conflicting views held by different investigators as to the nature of its attack on the host are well summarized by Brown [1] in a recent paper. In general, all writers agree on the presence of a cell-wall dissolving enzym, but differ widely as to the cause of the toxic action of the fungus.

As already stated, strawberries rotted by *Botrytis* sp. retain their shape, shrivel slightly, and even in a moist chamber there is no evident leaking. The moisture is apparently lost so slowly that it evaporates from the surface of the berry. A microscopic examination shows that the fungus has penetrated all parts of the berry; indeed, the cells are in many places embedded in the mass of mycelium and are apparently held together by it. The fungus is evidently capable of readily dissolving the middle lamella and of penetrating the cell walls themselves. Often hyphæ grow between the cells of the host for some distance and then penetrate the cells (Pl. XLIX, *A*). Not infrequently cells containing numerous hyphæ have the shrunken and distorted protoplasmic contents still present (Pl. XLIX, *B*, *C*, *D*). Sometimes hyphæ occur in adjacent cells whose separating wall remains intact and apparently unchanged (Pl. XLIX, B); or they may pass from one cell into the next, either where the cells are in contact or across an intercellular space (Pl. XLIX).

It is interesting to observe that hyphæ usually enter a cell at the angle where it joins two other cells; Plate XLIX, *D*, *F*, and *G*, shows examples. The hypha passes between two cells, apparently by dissolving the middle lamella, and then penetrates the wall of the cell with which it comes in direct contact. Occasionally a hypha seems to push back a portion of the cell wall before penetrating (Pl. XLIX, *G*). The fungus may, however, penetrate the wall at a considerable distance from the intersection of the cells (Pl. XLIX, *A*, *E*); or it may

[1] Brown, William. Studies in the physiology of parasitism. I. The action of Botrytis cinerea. *In* Ann. Bot., v. 29, no. 115, p. 313-348. 1915.

pass the point of intersection and penetrate a short distance beyond (Pl. XLIX, *H*).

Brown,[1] working with thin disks of tissue cut from various plants, particularly tubers of the potato and roots of the turnip, immersed in a strong extract from the germ tubes of *Botrytis cinerea*, noted that the separation of the cells followed the line of the cell walls, the cells on either side being left intact. His idea of the destruction of the cells is that the middle lamella is first dissolved, in consequence of which the tissue readily falls apart along the line of the middle lamella. Very soon the remainder of the cell wall disintegrates and the whole structure becomes very fragile.[2] In no case was complete solution of the cell wall observed. Death of the cells [3] takes place at a late phase in the process of disorganization of the cell walls. He observed also that in all cases if a cell wall was disintegrated death of the cell ensued; on the other hand, if the cell wall was not affected neither were the living contents of the cell.[4]

Brown's conclusions satisfactorily explain the condition found by the writer in strawberry cells attacked by *Botrytis* sp. Certainly the fungus is able to penetrate the cells of the host while they are still fairly normal in appearance and while the cytoplasm is still distinguishable (Pl. XLIX, *B*, *D*, *G*). The writer did not find, however, in any of the strawberries examined cells which were unaffected by the action of the fungus.

RESULTS OF INFECTION OF STRAWBERRIES BY RHIZOPUS SP.

In contrast to the condition of strawberries rotted by *Botrytis* sp., berries rotted by *Rhizopus* sp. show the following characteristics. The berries soon become flattened, with considerable loss of juice. Microscopic examination shows that the hyphæ are characteristically close to the surface of the berry, the majority being found in the outer six or eight cell layers. Hyphæ rarely or never penetrate the cells of the berry under field conditions or when kept in moist chamber. The nuclei of the cells persist in apparently normal condition until the cytoplasm of the cell has almost entirely collapsed.

The crowding of the fungus in the outer portion of the berry is very noticeable. Indeed hyphæ frequently grow for some distance immediately beneath the epidermis. Plate XLIX, *I*, shows a portion of such a hypha in a section cut nearly tangential to the surface of the berry. The small, thick-walled cells (heavy lines) on the right are epidermal cells; the larger, thin-walled cells (light lines) on the left are storage cells. The hypha, which could be traced across several sections, grows between these two layers of cells for a considerable distance without penetrating either. A similar condition is shown in vertical section in Plate XLIX,

[1] Brown, William. Op. cit., p. 333.
[2] Ibid., p. 335.
[3] Ibid., p. 347.
[4] Ibid., p. 345.

K, L. In the latter case the fungus has penetrated the epidermis and the external hyphæ are sporangiophores.

It is evident from a study of the sections that *Rhizopus* sp. does not readily penetrate the unbroken epidermis from the outside. Hyphæ are found which extend for some distance along the surface of the berry without penetrating. Plate XLIX, *J*, shows a portion of such a hypha; even the germ tubes seem unable to penetrate readily and often grow for some distance (Pl. XLIX, *M*) over the surface without penetrating.

Under field conditions or in moist chamber in the laboratory *Rhizopus* sp. apparently very rarely enters the host cells. Although several hundred slides were examined no single instance was found in which a hypha had penetrated a cell wall. Plate XLIX, *I-L*, shows that the hyphæ typically grow between the cells along the middle lamella. The effect of the fungus on the host cells is readily seen by the contraction of the protoplasm. Plate L shows strawberry cells in various stages of degeneration close to hyphæ of *Rhizopus* sp.

Plate L, *A*, shows the normal appearance of one of the smaller storage cells of the strawberry. In this case the cytoplasm contains numerous small vacuoles. Frequently, especially in larger cells, there is a single large vacuole. Plate L, *B*, shows a similar cell in which the protoplasm has begun to contract away from the wall. This cell was separated from the nearest hyphæ by three layers of cells. In Plate L, *C*, hyphæ of *Rhizopus* sp. are shown in contact with two host cells (a branch hypha overlies one cell). The protoplasm of these cells is much shrunken, but the cell walls retain their normal position, and the nuclei are unchanged. Plate L, *D*, *E*, *F*, and *G*, show progressively later stages in the breaking down of cells adjoining hyphæ. In some (Pl. L, *D*, *F*) the wall has begun to collapse. In all except Plate XLIX, *G*, in which there was very little cytoplasm remaining, the nucleus shows no signs of degeneration.

This persistence of the nucleus in apparently normal condition after the contraction of the protoplasm has progressed considerably is one of the most striking characteristics in berries attacked by *Rhizopus* sp. and is in sharp contrast to the condition found in berries rotted by *Botrytis* sp. Often in a cell in which the cytoplasm has largely disappeared and the wall is partly collapsed the nucleus appears large and typical, as in an intact cell (Pl. L, *J*). Frequently the cell wall collapses so rapidly that no space is left between it and the contracted protoplasm (Pl. L, *H*, *I*).

EFFECT OF RHIZOPUS SP. ON BERRIES IN EXTREMELY DRY AIR

In connection with experiments on the humidity relations of the fungus, berries inoculated with *Rhizopus* sp. were placed in a desiccator with concentrated sulphuric acid. Under these extremely dry conditions the berry "leaked" in the characteristic manner, but the habit of growth of the fungus was changed in two important particulars.

Fungus hyphæ were found in all parts of the berry, being abundant even in the center, within the circle of vascular bundles. Apparently the extreme dryness of the surrounding air made the intercellular spaces within the berry more favorable for its growth than the outer ones. Under these severe conditions the cells of the berry collapsed so generally that the relations of the fungus hyphæ to the walls could usually be studied only in cells near vascular bundles. It was evident that while, in general, the hyphæ grew between the cells of the host (Pl. L, *L*) they were frequently found inside the cells as well (Pl. L, *K*, *M*). It is worthy of note that in these berries several instances were found where hyphæ had punctured the cells and the nucleus of the cell was unchanged in appearance (Pl. XLIX, *K*).

COMPARISON OF THE FUNGI

The difference in the histological relations of the two fungi with the strawberry may be briefly summarized as follows:

Botrytis sp. penetrates all parts of the berry, growing within the cells as well as between them and ramifies through the tissues of the strawberry, surrounding and filling them with a network of mycelium. The cells of the berry seem to be quickly killed by the fungus; at least the protoplasm shrinks away from the cell wall and becomes disorganized so that no nucleus can be distinguished.

The mycelium of *Rhizopus* sp., on the other hand, is found chiefly in the outer portion of the berry. The hyphæ grow between the cells, separating them and apparently extracting the cell sap. The nuclei of the cells persist unchanged until a late stage in the breaking down of the cytoplasm. When the fungus is grown on berries in a dry atmosphere, its action is somewhat different. The mycelium penetrates to the center of the berry, and hyphæ are frequently found inside cells.

It is difficult to trace an exact causal relation between the histological differences in the attack of these fungi on the strawberry and the fact that they cause quite different types of rot. The fact that *Rhizopus* sp. separates the cells of the berries so completely may readily account for the berries affected with this fungus becoming so soft and easily flattened. On the other hand, the mycelium of *Botrytis* sp., by penetrating all parts of the strawberry, helps to hold it in shape and converts it into a mummy. It is possible that the juice of the berries affected by *Rhizopus* sp. is pressed out by the collapse of the berries, owing to the mere separation of the cells. This is, however, hardly an adequate explanation of the phenomenon.

While it is not proposed at the present time to review the rather voluminous literature on either of the fungi under consideration, a closely parallel case described by Behrens [1] should be mentioned in this

[1] Behrens, Johannes. Beiträge zur Kenntnis der Obstfäulnis. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 4, No. 12, p. 515-516. 1898.

connection. He observed in 1895 ripe tomatoes affected by *Mucor stolonifer* which reduced the pulp of the tomato to an almost fluid mass. A species of Fusisporium found at the same time on the tomatoes produced a dry-rot quite in contrast to the wet condition produced by the species of Mucor. Behrens found on microscopic examination that the mycelium of *Fusisporium* sp. penetrated the cells of the host, while the mycelium of *Mucor stolonifer* grew entirely in the intercellular spaces.

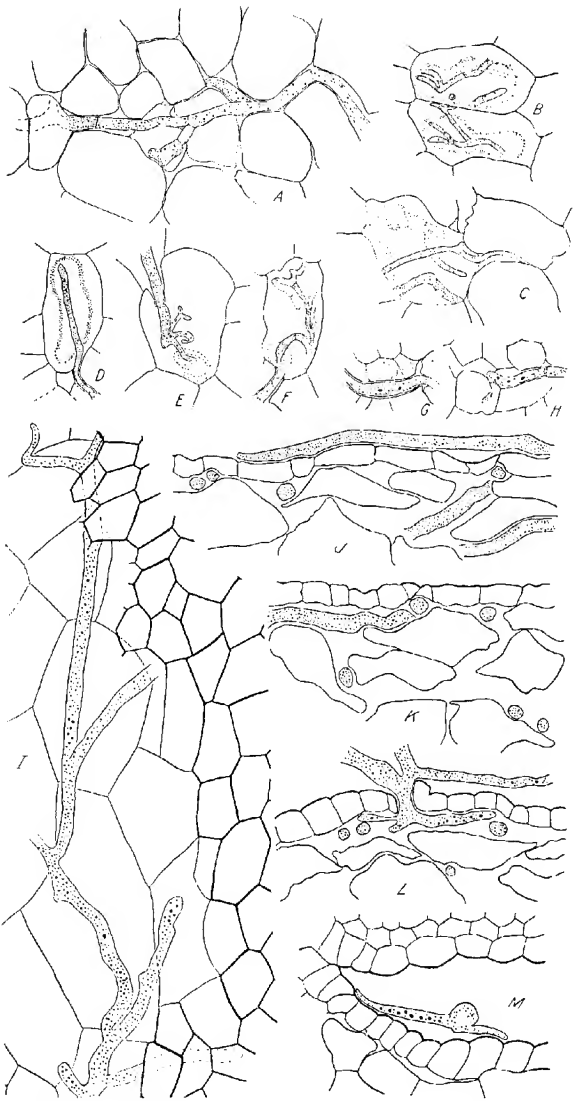
The relation of these fungi to each other in their attack on the berry is much clearer. In comparatively few cases have both fungi been found on the same berry and in no instance has the writer found a berry in which *Rhizopus* sp. had followed in a place originally infected by *Botrytis* sp.

Numerous cases have, of course, been found in which there were two fungi in the same berry; for instance, *Botrytis* sp. and *Fusarium* sp., *Botrytis* sp. and *Alternaria* sp., *Rhizopus* sp. and *Fusarium* sp. These fungi do not, however, seem to have entered in the same place, but rather from different portions of the berry. The mycelia of the two fungi sometimes mingle in the tissues of the berry—for example, *Botrytis* sp. and *Fusarium* sp., *Rhizopus* sp. and *Fusarium* sp.—or they may occupy different portions of the berry with a marked line of division between them, each apparently being unable to invade tissue occupied by the other fungus—for example, *Botrytis* sp. and *Alternaria* sp.

These observations do not preclude the possibility of *Rhizopus* sp. following in an area originally infected by *Botrytis* sp. or some other fungus, and this may occur in the field or in badly affected berries which are thrown out as culls in packing. They do, however, plainly indicate that *Rhizopus* sp. is not dependent on the presence of any other fungus in its attack on strawberries during shipment and on the market.

PLATE XLIX

A–H, Strawberry cells attacked by *Botrytis* sp. (× 210): *A*, Hypha growing partly between and partly within strawberry cells; *B*, hyphæ inside strawberry cells in which remnants of the protoplasm may still be distinguished; *C*, hypha passing from one cell into another across a short intercellular space; *D*, *E*, *F*, *G*, *H*, hyphæ entering cells in various ways (in *G* the hypha has pushed back a portion of the cell wall before breaking through). *I–M*, Strawberry cells attacked by *Rhizopus* sp. (× 210): *I*, Hypha growing between the epidermis and the adjacent layer of storage cells; *J*, hypha growing over the surface of the strawberry; *K*, hyphæ growing underneath the epidermal layer and between the storage cells; *L*, *Rhizopus* sp. growing between epidermal cells (basal portions of sporangiophores above and rhizoids below epidermis); *M*, germinating spore in cavity formed by a seed.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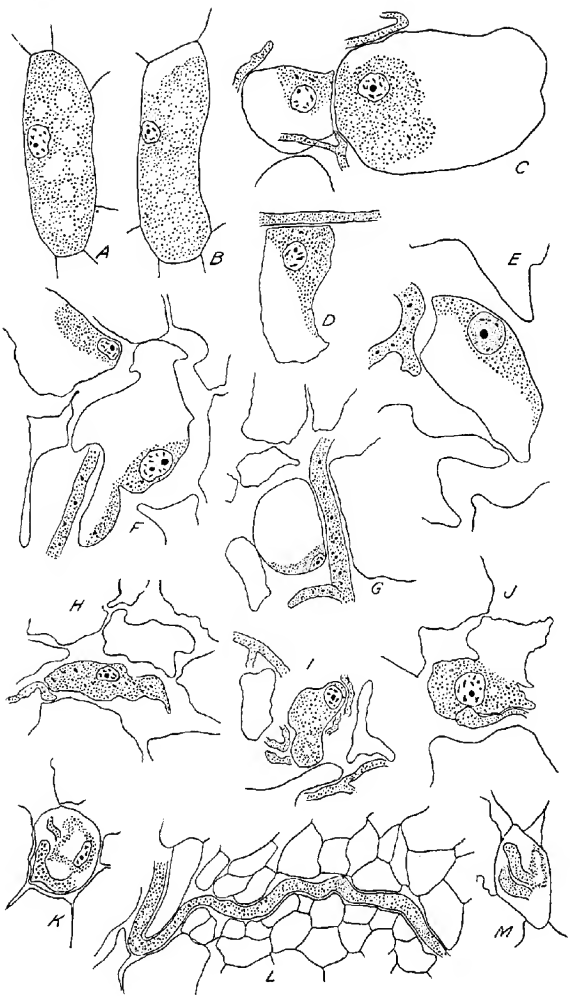
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PLATE L

Strawberry cells attacked by *Rhizopus* sp. *A*, Normal storage cell of strawberry; *B*, storage cell (near hyphæ) showing a slight contraction of the protoplasm; *C*, *D*, *E*, *F*, *G*, progressive contraction of protoplasm of host cells near hyphæ (the cell walls have contracted very little); *H*, *I*, *J*, strawberry cells near hyphæ in which the cell wall has crumpled with the contraction of the protoplasm; *K*, *M*, hyphæ inside cells; *L*, hyphæ growing between cells of the strawberry; *K*, *L*, *M* are drawn from berries which had been rotted in the desiccator. (× 210.)

LIFE HISTORIES AND METHODS OF REARING HESSIAN-FLY PARASITES

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INTRODUCTION

The most effective factors in the control of the Hessian fly (*Mayetiola destructor* Say) in the past have been its parasites. There are seasons, however, when the parasites become scarce and the Hessian fly exceedingly abundant. Again, in the same season the Hessian fly seems practically free from parasites in some localities while in others its parasites are numerous. A thorough knowledge of the life histories, field habits, relative efficiency, and effective methods of artificial propagation and dissemination of the different parasites, therefore, might make it practicable to introduce the most efficient species from localities where they are abundant into other localities where the host is working destruction unchecked by its enemies. It might also be possible to propagate artificially and to disseminate the parasites during periods when they have become scarce in the fields, and thereby shorten the period of destructive abundance of the Hessian fly. Up to the present time very little accurate and detailed information seems to have been recorded regarding the life stages, habits, and efficiency of Hessian-fly parasites. It has been uncer tain whether or not some of the species involved were true parasites. Some results in this direction have been accomplished by the author during the last two seasons, and the purpose of this paper is to make public these results and the methods used in attaining them.

The life histories and methods of rearing three hymenopterous parasites are treated in this paper: *Eupelmus allynii* French, *Merisus destructor* Say, and (*Merisus*) *Micromelus subapterus* Riley. The seasonal history and field habits of these parasites will require another season's observation before they can be effectively treated. The scope of this paper is therefore limited to the life histories and relationships of these species to one another and to their common host as determined under laboratory conditions.

[1] The writer wishes to acknowledge his indebtedness to Messrs. E. O. G. Kelly, W. R. Walton, A. B. Gahan, W. R. McConnell, and J. A. Hyslop, all of the Bureau of Entomology, for helpful advice; to Mr. Kelly for making the work possible, and to Mr. Gahan for determining all specimens.

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METHODS OF BREEDING AND REARING

The adult parasites used in all experiments were kept in modified forms of the Doten cage.[1] One form, used when it was desired to confine a number of parasites together, consisted of two large, straight-sided vials of the same diameter, the mouths of which snugly fitted into a paper tube 1 inch long. This paper tube was held in shape by a layer of adhesive plaster around the outside. The cage was prevented from rolling by sticking a square of heavy cardboard to one side of the connecting tube. A label was pasted to the upper side of the tube for identification. One vial was kept dry and clean, while water and honey were supplied in the other.

The other form of Doten cage, used chiefly for isolating pairs and individuals, was simply a small, straight-sided vial into the mouth of which was fitted the open end of a slightly smaller, straight-sided vial. A small label was pasted on the side of the larger vial for identification. Cages of this kind were prevented from rolling by keeping them in shallow boxes with corrugated pasteboard-lined bottoms. Food and water were placed in the smaller vial.

In both forms of cages the water and the honey used for food were placed separately in small droplets on the upper surface inside the food vial. The honey used was the extracted form diluted with an equal amount of water. It was necessary to exercise considerable care not to place too large a drop of honey in a cage, because of its tendency to run down on the inside of the vial and to entangle the insects. Fresh water and honey were placed in the cages daily, and at least once a week the food vials of the cages were carefully cleaned to remove dried or soured honey. Replenishing the food and water in the cages once a day seemed sufficient to supply the needs of the parasites. It was often found necessary to make up a fresh supply of the honey because of souring or molding, especially in hot weather. Sterilizing the fresh supply by placing the dropper bottle containing it in boiling water for a few minutes caused it to remain sweet and usable much longer.

BREEDING THE PARASITES

To determine all the life stages from egg to adult involved the processes of exposing Hessian-fly puparia to parasites, dissecting the parasite eggs from the host puparia, and rearing, in little glass-cell cages devised for the purpose, the resulting parasite larvæ on Hessian-fly larvæ which were also dissected from puparia. Hessian-fly puparia contained in sections of wheat stems were first exposed to the adult parasites by placing the stems in the vial cages containing the adults. The stems remained in the cage for a day, or until a parasite was seen to oviposit in a flaxseed, when they were removed and the puparia dissected. The

[1] Doten, S. B. Concerning the relation of food to reproductive activity and longevity in certain hymenopterous parasites. Nev. Agr. Expt. Sta. Tech. Bul. 78, 30 p., 10 pl. 1911.

eggs of the three species studied were always found between the inner surface of the puparium and the larva itself. They were transferred separately, each to an unparasitized Hessian-fly larva which had been previously dissected from its puparium and placed in a little glass-cell cage of the following description:

Flat glass plates 1 inch by 1¼ inches square were used, in which hollows about the size and shape of a Hessian-fly puparium were ground in one surface, one hollow per plate, this work being done with a small carborundum grinder. After a host larva and a parasite egg had been placed in a hollow, the cell was closed by covering it with an ordinary glass cover slip. The cover glass was held in place by two little dabs of honey on its underside. The cell was not sealed by a complete ring of the adhesive because of the desirability of diffusion of atmospheric moisture under the cover slip. Honey seemed to be the ideal adhesive for this purpose, since it had no odor harmful to the inmates of the cell; it held the cover-glass tight against the slide; it did not dry so hard as to prevent the cover-glass from being easily removed; and a supply of it was always convenient. A label was pasted on the glass plate near one end for identification. The complete development of the parasite from egg to adult on its host could then be observed under the binocular in this little cell without disturbing the parasite or the host in the least.

With each of the three species the period from oviposition to emergence of adult, when individuals were reared in glass cells, approximated very closely the period from egg to adult when individuals were reared under the same meteorological conditions in Hessian-fly flaxseeds. Hence, the length of each stage of development as determined from individuals reared in glass cells may be considered normal.

It was discovered that the larvæ of all three species molted while making their growth within the little cells. The length of the instars was not observed, but the number of molts was determined by transferring to a balsam mount on a microscope slide all the material left behind in the little glass cell where a single individual had made its growth. In cases where the larva had pupated, the last molted skin was added to the mount. In cases where the full-grown larva had not pupated, the mandibles borne by the larva were dissected from it and added to the mount. To determine the number of molts of a single individual, the mount of the material it left behind was examined under the microscope and the number of pairs of mandibles in the material ascertained. In all cases the cell in which the larva made its growth was known to be absolutely clean when the host and the egg from which the parasite larva hatched were placed in it; hence, it was known that all pairs of mandibles found in a mount belonged to the same larva. Cells which contained simultaneously the remains of more than one parasite larva were not used in determining the number of molts. No attempt was made to determine the number of molts of individuals which had made their growth inside flaxseeds.

EUPELMUS ALLYNII

THE EGG

The egg of *E. allynii* French (Pl. LI, fig. 1) is elliptical in shape, with a thin stalk of varying length on one or both ends. In some cases the stalk seems to be entirely absent from one end. The egg is grayish white in color. The long axis of the body of the egg averages 0.35 mm., the short axis 0.14 mm. in length. As a parasite of the Hessian fly, the observations at Wellington, Kans., indicate that the egg is normally deposited in the puparium of the host. Females were repeatedly observed by Mr. E. O. G. Kelly and the author to be very numerous in fields, ovipositing in Hessian-fly flaxseeds where these constituted the only stage of the fly to be found. In one instance, however, a wheat stem containing nearly grown Hessian-fly larvæ, but no flaxseeds, was placed in a vial cage containing females of *E. allynii*. Upon dissecting this stem two eggs of this parasite were found inside the leaf sheath close beside the Hessian-fly larvæ. Whether or not the parasite is able to complete its development on Hessian-fly larvæ before they have formed puparia is still unsettled.

Hundreds of flaxseeds in which *E. allynii* had oviposited have been dissected and the eggs of the parasite have always been found inside the puparium but external to the inclosed Hessian-fly larva or pupa. Sometimes they were unattached, but more often the egg was fastened to the inner surface of the puparium by a little netlike structure made apparently of fine, white threads tangled together (Pl. LI, fig. 2). The threads forming the net appeared to be identical in diameter, color, and material with the egg stalks. The edges of this little net or mat were fastened down all around the egg, holding it securely in place. Sometimes the net was partly fastened to the host larva in addition to the puparium. In all experiments *E. allynii* oviposited seemingly indiscriminately in flaxseeds already containing parasite larvæ as well as in those containing Hessian-fly larvæ. The incubation periods of 109 eggs varied from 1½ days to 5 days. The egg stage was shorter in summer temperatures, observations being made during a period from July to November.

THE LARVA

Upon becoming fully formed inside the egg the larva (Pl. LII, fig. 2) breaks through one end of the chorion and after crawling around a little attaches itself to the external surface of the host larva. The parasite larva bears strong mandibles and feeds externally on the Hessian fly by puncturing the epidermis of the host and sucking out the body liquids. Larvæ reared in glass cells became full grown in from 7 to 10 days. After becoming full grown many of the larvæ were inactive for months; others pupated at once. In the warm summer temperatures most of the larvæ reared pupated at once upon completing their growth, while larvæ reared in the fall pupated only in occasional instances.

The larvæ reared in glass cells normally pass through five instars. Nearly all mounts made of the material left behind by larvæ which had finished feeding showed a total of five pairs of larval mandibles, while in the remaining mounts from two to four pairs were found which always correspond in size and shape to some one pair in the complete series. Five was the maximum number found in any one instance, and in cases where less than five were present it appeared that some of the molts had been lost in manipulation. Where five pairs of mandibles were found in a single mount, the sizes increased fairly uniformly from the second molt to the last. The mandibles and head shields of newly hatched larvæ appeared to be more heavily chitinized than those of later instars, except the last, and somewhat larger than those of the second instar. The mandibles of all instars are similar in shape. They articulate laterally with the head and fold together across the mouth, the ends overlapping. They are decidedly curved, taper to points, and are brown and chitinous. The sharp distal portions of the mandibles enlarge suddenly into a comparatively broad base bearing a chitinous lobe on the ventral side. The following average measurements will show the relative sizes of molted mandibles. These measurements represent the distance in a straight line, from the tip of the mandible to the shoulder, where the mandible suddenly enlarges into the broad basal portion.

Molt No.	Length of mandible.
1	0.016 mm.
2	.016 mm.
3	.024 mm.
4	.032 mm.
5	.048 mm.

The full-grown larva is grayish white, averaging about 3 mm. long and 0.9 mm. in diameter, with 13 body segments besides the head. There are no tubercles on the head, but there is a row of four hairs evenly spaced across the top. The front of the head bears a pair of hairs, one on each side, just outside of each of which is a very short, white, conical projection, apparently antennæ. There is a short bristle near the base of each mandible. The mouth is chitinized along its upper edge, this brown, chitinous rim extending around the bases of the mandibles and bearing six toothlike lobes pointing downward along the portion of the edge between the mandibles (Pl. LII, fig. 1). A subdorsal and sublateral row of fine, white hairs runs the full length of the body on each side, one hair per segment in each row. The first three body segments bear several additional rows. What appears to be the anal segment is divided into a dorsal and a ventral lobe by a transverse invagination across the end. The dorsal lobe bears two pairs of short, fine hairs, one pair close together near each lateral end of the lobe. The ventral lobe bears a short hair at each lateral end. The body hairs are evidently tactile organs, since when any of them are touched, the larva wriggles and bites viciously at the point of contact.

Larvæ of this species seem to be better equipped, more vigorous, and more capable of defending themselves than the larvæ of *Micromelus subapterus* and *Merisus destructor*. *E. allynii* was reared from egg to adult on larvæ of both the other species just mentioned as well as on the Hessian fly. In one case, however, a newly hatched larva of *E. allynii* placed on a full-grown larva of *M. subapterus* in a glass cell was killed by the latter almost immediately. A few instances were observed where larvæ of *E. allynii* killed other individuals of the same species present in the same Hessian-fly puparium.

THE PUPA

The larva forms a naked pupa (Pl. LI, fig. 3, 4) inside the puparium of the host. The first step in the process is the excretion of all waste matter from the body, leaving the larva pure white. The pupa is then formed and the last larval skin cast off. The newly formed pupa is nearly white, but turns dark within a few hours. The pupal stage of 30 specimens reared in glass cells varied from 9 to 24 days. The pupal period of those pupating in the summer averaged 13 days, while the pupal periods of those reared late in the fall became as long as 24 days in some cases. The arrival of cold weather retards pupal development, but whether or not the pupæ are able to survive severe winter temperatures has still to be determined. When the adult has completely developed, the pupal skin is cast off inside the host puparium, and the adult gnaws a round hole through the flaxseed near one end, penetrating the leaf sheath covering the flaxseed, through which it emerges.

THE ADULT

After remaining quiet until dry, the adult becomes very active. Adults do not seem to fly more than a few feet at a time, using their wings merely to go from stem to stem. They do this so quickly and often that it is difficult to observe a single individual in the field very long. The females run quickly up and down the wheat stems, vibrating their antennæ rapidly against the side of the stem until they come to a place where a Hessian-fly puparium is located. Here they feel back and forth above the flaxseed until they locate the exact spot which suits them for oviposition. Then, facing upward, the tip of the abdomen is bent down until it touches the stem and raised away again, leaving the ovipositor pressed vertically against the stem supported from its articulation with the middle ventral portion of the abdomen. The leaf sheath and puparium are pierced by what under the microscope appears to be a sort of drilling motion of the ovipositor, which seems to be rotated part way around and back again. Oviposition takes several minutes.

Males placed in the same cage with females usually attempt to mate with them at once. Mr. W. R. McConnell has ascertained that this species can reproduce parthenogenetically. The question of the sex

of parthenogenetic progeny has not yet been definitely settled. Mated females produced both male and female progeny. Two mated adults kept separately in vial cages from the time they emerged from pupæ until they died each laid a total of 58 eggs. This number actually was found in each case by dissection of flaxseeds which had been exposed to the adult. A few eggs may have been lost in dissection. These adults remained alive for periods of 48 and 56 days and were ovipositing during periods of 29 and 46 days, respectively. Another adult, caught in the field while ovipositing in a flaxseed, remained alive in a vial cage and oviposited in flaxseeds during a period of 57 days. An unmated female was kept alive in a vial cage for 83 days. How long adults normally live in the field is not known.

In one experiment Mr. W. H. Larrimer, of the Bureau of Entomology, exposed stems of *Elymus canadensis* containing galls of *Isosoma* sp. to two *Eupelmus allynii* females which previously had been ovipositing in Hessian-fly puparia. They at once oviposited in the galls. The galls were dissected and the inclosed larvæ of *Isosoma* sp., together with the eggs of *E. allynii* found in the galls, were transferred to glass-cell cages, one larva of *Isosoma* sp. and one parasite egg to each cell. The parasites proceeded to complete their development to adults on the larvæ of *Isosoma* sp. Progeny were also bred on the Hessian fly from the same parents used by Mr. Larrimer. These parents and their progeny were all determined by Mr. Gahan as *E. allynii*.

MERISUS DESTRUCTOR

THE EGG

The egg of *Merisus destructor* Say (Pl. LI, fig. 5) is elongate, kidney-shaped, circular in cross section, with one end smaller than the other. It is white, with the surface apparently smooth. The average length of eggs measured was 0.4 mm., the average diameter at thickest point, 0.1 mm. Hundreds of the eggs were dissected from flaxseeds, in which they had been deposited, and in all cases they were found external to the host larva or pupa inside the puparium. Some eggs apparently bore a short pedicel on one end, which seemed to be fastened to the inside of the host puparium. Ordinarily, however, the eggs were found free.

M. destructor, like *E. allynii*, normally oviposits in the Hessian-fly flaxseed, according to the observations of Mr. Kelly and the author at Wellington, Kans. It was very abundant in the fields at times when no other stage of the Hessian fly was present. The females were repeatedly observed ovipositing in puparia in the field. In cages they also oviposited readily in flaxseeds contained in sections of wheat stems as well as in naked flaxseeds removed from stems. They did not oviposit readily in sections of stems containing only partially grown Hessian-fly larvæ, although they seemed interested in them. In one instance,

however, a female *M. destructor* oviposited in a stem containing nothing but partially grown larvæ. Upon dissection the egg was found sticking to the stem underneath the leaf sheath, close to one of the larvæ. It is not yet known whether or not *M. destructor* can develop to maturity on partially grown Hessian-fly larvæ. The egg stages of 96 specimens placed on Hessian-fly larvæ in glass slides varied from 1½ days in hot July weather to 4 days in cool September weather. The larva emerges from the egg by breaking through one end. After crawling around a little the larvæ reared in glass cells fastened themselves with their mandibles to the outside of the host larvæ in order to feed.

THE LARVA

The full-grown larva of *M. destructor* (Pl. LII, fig. 4) is white with the dingy brown contents of the alimentary tract visible through the integuments. There are two pairs of slightly raised circular tubercles on the front of the head near the top. The lower pair are slightly farther apart than the upper pair and each bears a small conical projection, evidently an antenna, varying from white to pale brown in color and about 0.02 mm. long. The median ventral surface of the head bears the round suctorial mouth opening. The only mouth appendages distinguishable are a pair of brown chitinous mandibles borne laterally and closing together across the mouth with their tips overlapping (Pl. LII, fig. 3). The distal portion of the mandible is conical, tapering gradually to a sharp point. The proximal end is suddenly enlarged, evidently to provide for muscle fastenings. One subdorsal and one sublateral row of very short and inconspicuous setæ on each side of the body are clearly distinguishable in some specimens, extending the full length of the body, one seta per segment in each row. On some specimens there appear to be two ventral and two dorsal rows of scarcely discernible setæ on the first three body segments only. There are thirteen body segments besides the head, the anal segment being divided into a dorsal and a ventral lobe by a horizontal fold across the end. The dorsal lobe bears four very short, fine setæ in a row across the end, the setæ composing the row being usually in two lateral pairs. The ventral anal lobe bears only two setæ, one near each lateral end of the lobe. The length of the full-grown larvæ averages 2.5 mm., the largest diameter, 0.7 mm.

Balsam mounts of all the material left behind in the little glass cells by pupating larvæ nearly always contained five pairs of mandibles. Mounts of all the material left in the cell by full-grown larvæ which had ceased to feed, together with the mandibles dissected from such larvæ, also nearly always contained five pairs of mandibles. In every mount the pairs varied uniformly in size from those resembling the ones borne by newly hatched larvæ to those borne by full grown larvæ. Mandibles of newly hatched larvæ were somewhat hooked. All the remaining pairs were similar in shape, and corresponding pairs in all the mounts

were almost identical in size. As was the case with *E. allynii*, the mandibles of the newly hatched larva appeared to be heavier, more powerful, and somewhat larger than the mandibles borne by the second-instar larva. Also, the head shield appeared to be more heavily chitinized in the first instar than in the later ones. Beginning with the second instar, the successive pairs of mandibles apparently increase fairly uniformly in size with each molt. In the mounts where five pairs of mandibles could not be found, those which were found correspond in size and shape to some one of the pairs in the complete series and it was evident that certain pairs had been lost in making the mount. No more than five pairs were found in any one case. All the findings lead to the conclusion that larvæ of *M. destructor* normally pass through five instars in making their growth.

The relative sizes of the molted mandibles are shown below. The measurements represent the distance in a straight line from the tip of the mandible to the shoulder where it suddenly enlarges into the broad base.

Molt No.	Length of mandible.
1	0.014 mm.
2	.014 mm.
3	.020 mm.
4	.024 mm.
5	.032 mm.

The larvæ develop readily on Hessian-fly larvæ and pupæ, both in flaxseeds and in glass cells, unless the host pupa has nearly completed its development. Several newly hatched larvæ in flaxseeds and glass cells containing Hessian-fly pupæ which were nearly developed killed the pupæ, but died from lack of sufficient food to complete their growth. The larvæ are evidently cannibalistic upon occasion. In one flaxseed which had been exposed to ovipositing females, a young larva of *M. destructor* was found which had been feeding, as also the shrunken remains of another young larva. Evidently the healthy larva had found and killed the other and was feeding on the Hessian-fly larva when the flaxseed was dissected. Full-grown larvæ in glass cells punctured and killed eggs and larvæ of *M. destructor* which were placed in the cells with them. Larvæ of *M. destructor* were able to become full grown by feeding on larvæ of *M. subapterus* also.

The periods required by 36 larvæ to make their growth when reared in glass cells varied from 7 to 11 days. Cool weather appeared to make growth slower. After becoming full-grown the majority of the larvæ of *M. destructor* reared in glass cells remained quiescent for months, though still alive and able to wriggle vigorously when touched. Larvæ reared in flaxseeds exhibited the same characteristic. In other words, the larvæ seem to have a tendency to estivate and hibernate until another warm season before pupating. Larvæ of *M. destructor* were actually found to

have hibernated in stubble of wheat cut the previous June. Eight per cent of the flaxseeds in stubble gathered from a field in southeastern Kansas in late March contained live, full-grown parasite larvæ which afterwards became adult and were determined by Mr. Gahan as *M. destructor*.

THE PUPA

The period from the formation of the pupa (Pl. LI, fig. 6) to the emergence of the adult varied from 7 to 14 days in 21 specimens carried through this stage in glass cells. Those pupating in April and September, when cooler temperatures prevailed, took longer to develop than those which pupated during the hot weather of July and August. The larvæ form naked pupæ inside the puparium of the host. The process of pupation as observed in glass cells begins with the excretion of all waste matter from the body of the larva, which then becomes pure white. In a few hours the pupa is formed. The eyes begin to turn reddish in about a day and by the fourth day are a very dark red. The body of the pupa is by the fourth day a creamy white, and by the sixth day the head and thorax are black. Within another day the abdomen turns black except for the base of the abdomen, which assumes the light brown as found in males and some females. The emergence of the adult follows within a day or so after the pupa has turned dark. Cool weather retards development. The adult casts off the pupal skin inside the host puparium and emerges by gnawing a round hole through the side of the flaxseed and the wheat leaf sheath covering it just large enough for the adult parasite to crawl through.

THE ADULT

Adults soon become active after emerging from flaxseeds. In the spring males emerged two or three days before the females in cages containing stubble collected from the fields where it had stood during the winter. Mating took place at once when the females emerged. Oviposition takes place in the following manner: The females run up and down the wheat stalks, vibrating their antennæ rapidly against the side of the stem. When they come to a place where there is a flaxseed underneath the leaf sheath, they stop and excitedly feel up and down over the place where the flaxseed is located. They face upward to oviposit, with the body parallel to the puparium. They locate the proper place for oviposition with the tip of the abdomen and then raise it away from the stem, leaving the ovipositor unsheathed and pointing perpendicularly against the stem from its articulation with the middle of the abdomen. In less than a minute the ovipositor is forced through the leaf sheath and the puparium. In penetrating the flaxseed the ovipositor is seemingly rotated like a drill part way round and back again. Oviposition takes 5 to 10 minutes, and dissections of flaxseeds indicate that a single egg is laid at a time. One female kept isolated in a vial cage laid a total of 39 eggs in puparia exposed

to her and later dissected. Some may have been lost in dissection. This female was laying eggs during a period of six weeks. Other females were kept alive and active in confinement for periods of over two months.

Some stems of *Elymus canadensis* containing galls formed by a species of Isosoma were placed in a vial cage containing females of *M. destructor*. Almost immediately one of the females became interested in the galls, feeling over them with her antennæ. She then attempted to oviposit, endeavoring persistently to penetrate the gall with her ovipositor, but without success. Mr. W. H. Larrimer finally succeeded in getting the females to oviposit in the Isosoma galls and found the eggs inside the galls but external to the larvæ of *Isosoma* sp. He actually reared a few specimens of *M. destructor* from egg to adult on the Isosoma larvæ in glass cells. The parents used in this experiment and the progeny which were reared were determined as *Merisus destructor* by Mr. Gahan.

MICROMELUS SUBAPTERUS[1]

Heretofore it has been uncertain that the winged and wingless forms of *Micromelus subapterus* Riley were the same species. It has been proved, however, that the two forms are specifically identical by breeding a wingless female from a winged parent. Further evidence indicating that the winged and wingless forms are the same species is the fact that wingless males mated with winged females as readily as with the wingless form. The method by which the wingless female was bred from the winged parent is as follows: The winged parent deposited an egg in a Hessian-fly puparium known to have been previously unparasitized. The egg was removed from the puparium and from it a wingless adult was reared on a healthy Hessian-fly larva, which also had been dissected from its puparium. Mr. Gahan found this wingless offspring of a winged adult to be identical with winged specimens of unknown parentage.

THE EGG

The egg of *Micromelus subapterus* (Pl. LI, fig. 7) resembles that of *Merisus destructor* in size and shape. It is elongate, kidney-shaped, with one end longer than the other, circular in cross section, white in color, with surface of shell smooth, and about 0.38 mm. long by 0.09 mm. in diameter at the thickest point. It has no stalk.

All the observations made at the Wellington (Kans.) station lead to the conclusion that the egg is normally laid in the Hessian-fly puparium. In cages the adults oviposit readily in flaxseeds, the eggs being placed inside the puparia but external to the inclosed Hessian-fly larvæ and unattached. This was the case both when stems of fly-infested wheat

[1] Mr. A. B. Gahan makes the following statement: "The real generic position of this species is in doubt. It was originally described by Riley under the name *Merisus* (*Homoporus*) *subapterus* Riley, and later referred to Bœotomus by Osborn and other writers. N. V. Kurdiumov has more recently placed the species in the genus Micromelus. Doctor Ashmead reduced Bœotomus to synonymy with *Micromelus*."

were exposed to the parasite in vial cages and when ovipositing females were placed in large glass chimneys containing growing wheat infested with the Hessian fly. Occasionally the females have been observed apparently to oviposit in stems containing only larvæ; and although careful dissections of these stems were made, no eggs were found. Further proof that *M. subapterus* normally oviposits in flaxseeds was obtained by dissecting puparia collected in fields where this parasite was numerous at the time the collection was made. Both eggs and young larvæ of a parasite were present in the flaxseeds and when reared to maturity in the laboratory were found to be *M. subapterus*. The egg stage in 119 cases varied from 1¼ to 5 days. Low temperatures in fall and spring retarded embryonic development. The larvæ reared in glass cells emerged from the eggshells by breaking through one end, and after crawling around a short time settled down in one place to feed.

THE LARVA

The full-grown larva of *M. subapterus* (Pl. II, fig. 6) averages 2 mm. long by 0.75 mm. in thickness. It is white, with the pale-brown contents of the alimentary tract showing through the body. There are two pairs of slightly raised circular tubercles on the front of the head near the top. The lower pair are slightly farther apart than the upper pair, the former each bearing a small conical projection, evidently the antennæ, varying from white to brown and about 0.015 mm. long. The median ventral surface of the head bears the round, suctorial mouth opening. The only mouth appendages distinguishable are a pair of very small brown chitinous mandibles borne laterally and closing together across the mouth (Pl. LII, fig. 5). The distal ends of the mandibles are sharp and needlelike. The proximal ends are suddenly enlarged, evidently to provide for muscle fastenings. A minute pit, which sometimes appears to have a brown center, occurs on each side of the mouth. The body is entirely glabrous, so far as could be determined, except for the anal segment, oval in shape, with the anal end the more pointed. There are 13 segments besides the head, the anal segment being divided into a dorsal and ventral lobe by a horizontal fold across the end. The dorsal lobe bears four short, very fine setæ in a transverse row, these usually being in lateral pairs. The ventral anal lobe bears only two very short, fine setæ, one near each lateral end of the lobe.

The number of instars passed through by larvæ of *M. subapterus* in making their growth appeared to be five. Five pairs of molted mandibles increasing uniformly in size, from the small pair resembling those borne by newly hatched larvæ to the large pair molted off when full-grown larvæ pupated, were present in almost every mount made of the material left behind in a cell where a larva had developed. In

mounts where five pairs could not be found, each of those present corresponded to some one of the pairs in the complete series. No more than five pairs were found in a single mount. As in the two species of parasites previously discussed, the head shield of the newly hatched larva was more heavily chitinized than those of later instars. The mandibles appeared to be more powerful for their size than those of any later instar, and in some cases they were actually larger than the second-instar mandibles. The approximate sizes of the respective pairs of molted mandibles follow. The measurements represent the distance from the tip of the mandible to the shoulder where it suddenly enlarges into the broad base.

Molt No.	Length of mandible.
1	0.012 mm.
2	.012 mm.
3	.016 mm.
4	.020 mm.
5	.028 mm.

Larvæ of *Micromelus subapterus* do not seem as capable of moving around and reattaching themselves to the host as are the larvæ of *Eupelmus allynii* and *Merisus destructor*. Larvæ reared in glass cells crawled about a little immediately after hatching before they settled down to feed, but they usually completed a large part of their growth without leaving the original feeding point on the external surface of the host.

This species not only developed on Hessian-fly larvæ in puparia, but in some instances fed on the larvæ of other parasites. One egg of *Micromelus subapterus* was placed on a full-grown larva of the same species in a glass cell. The egg hatched and the little larva became full grown on the large larva, almost completely devouring it. Another egg of *M. subapterus* was placed on a full-grown larva of *Merisus destructor* and the little larva hatching from the egg became full grown on the larva of *Merisus destructor*. Experiments like these, however, usually resulted in the destruction of the egg or young larva of *M. subapterus* and the survival of the full-grown larva of the same or the other species as the case happened to be. Larvæ of *M. subapterus* apparently could make their growth on the Hessian-fly pupa as well as on the larva unless the former had partially developed. Where the host pupa had already completed a large part of its development, both the host and the parasite generally died, the latter apparently for lack of sufficient suitable food. Larvæ of *M. subapterus* appeared to be the least able to defend themselves where the larvæ of more than one species occurred in the same flaxseed. They also seemed the least capable of successfully establishing a feeding point on the host larva, at least when reared in little glass cells. They seemed more delicate in structure and less vigorous.

The respective periods required for 36 larvæ to make their growth varied from 7 to 10 days. A large proportion of the larvæ after finishing

their growth remained in a quiescent state in the little glass cells for months. Others pupated at once upon completing their growth.

THE PUPA

In general, the process of pupation as observed in glass cells is as follows: The full-grown larva excretes all waste matter from the body, leaving it perfectly white. Within a day after this operation the pupa (Pl. LI, fig. 8) is formed and is at first perfectly white, the last larval skin being found at the anal end of the pupa. In another day or so the pupa begins to turn a pale brown, and the eyes turn reddish. The pupa finally becomes entirely black as development progresses, the head and thorax changing first, and remains so until the adult emerges.

The pupa is formed naked inside the puparium of the host. The adult emerges by casting off the pupal skin inside the host puparium and then cutting a round hole through the side of the flaxseed near one end. The length of the pupal period varied in 21 instances from 7 to 13 days. Cool weather retarded the development of the pupæ. A larger proportion of the larvæ reared in the cooler weather of fall pupated at once upon attaining their growth than was the case with the larvæ reared in the hot weather of midsummer, indicating a tendency of the larvæ of this species to estivate.

THE ADULT

Newly emerged adults became active almost at once upon emerging from the host puparium. Males placed in the same cage with females began mating at once. Females that had been mated seemed to oviposit more readily than unmated females. Both Mr. McConnell and the writer found that this species was arrhenotokous in every instance where this point was determined. Females have been kept alive in cages as long as six months, and one female oviposited after having been kept alive over five months. It was usual for them to live and oviposit for at least a month in vial cages. One female actively oviposited during a period of 75 days and laid a total of 103 eggs. Another female laid a total of 45 eggs. The number of eggs laid by a single female was determined by exposing flaxseeds to an isolated individual and dissecting them to find the number of eggs the parasite had laid in each.

In ovipositing the female would run up and down the stems of the plants, vibrating her antennæ against the surface. When she came to a place in the stem where a flaxseed was located, she would stop, feel up and down over the spot with her antennæ, and then lower the tip of her abdomen. When she had found the point that suited her for oviposition, the end of the abdomen was raised, leaving the ovipositor standing vertically against the side of the stem from its articulation with the middle of the abdomen. In penetrating the leaf sheath and puparium the parasite seemed to rotate the ovipositor with a drilling motion in

addition to the downward pressure exerted on it. The female always took a position heading up the stem in ovipositing. The whole process generally took five minutes or more.

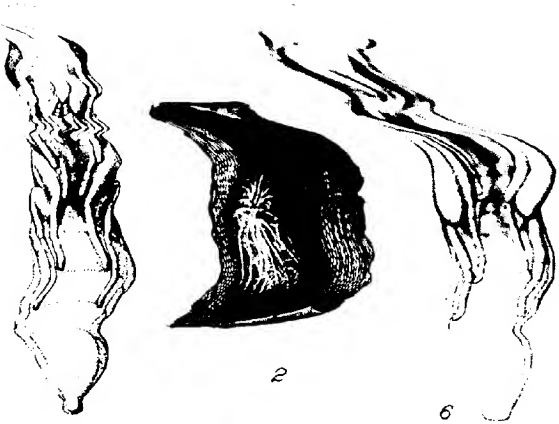
CONCLUSION

The writer's experiments and observations have all led to the inference that only one specimen of any of the three species studied ever matures in a single Hessian-fly puparium. In every instance where more than one egg or larva was placed on the same host or in the same cell, one survived and the rest were killed by that one, or starved to death. This was true whether the two or more larvæ were of the same or different species.[1]

[1] For correct figures of the adults of all three of the species treated in this paper, see U. S. Dept. Agr. Farmers' Bul. 640. (Webster, F. M. The Hessian fly. 20 p., 17 fig. 1915.)

PLATE LI

Fig. 1.—Egg of *Eupelmus allynii*.
Fig. 2.—Egg of *Eupelmus allynii* in situ.
Fig. 3, 4.—Pupa of *Eupelmus allynii*.
Fig. 5.—Egg of *Merisus destructor*.
Fig. 6.—Pupa of *Merisus destructor*.
Fig. 7.—Egg of *Micromelus subapterus*.
Fig. 8.—Pupa of *Micromelus subapterus*.



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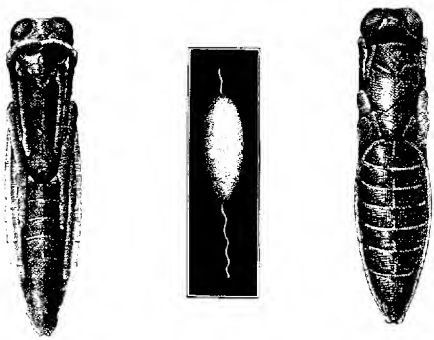

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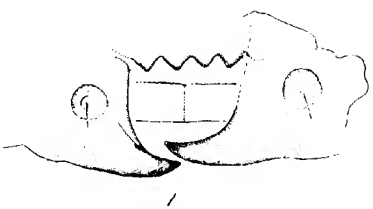

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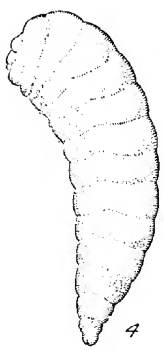

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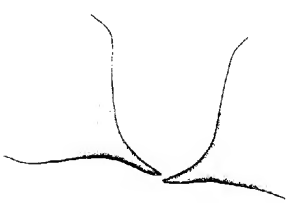

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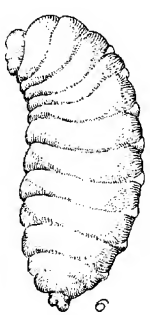

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PLATE LII

Fig. 1.—Mandibles of full-grown larva of *Eupelmus allynii*.
Fig. 2.—Larva of *Eupelmus allynii*.
Fig. 3.—Mandibles of full-grown larva of *Merisus destructor*.
Fig. 4.—Larva of *Merisus destructor*.
Fig. 5.—Mandibles of full-grown larva of *Micromelus subapterus*.
Fig. 6.—Larva of *Micromelus subapterus*.

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